

BOUND BY
BURGMEIER
BOOK BINDERY, INC.
CHICAGO 6, ILL.



PRESBYTERIAN-ST. LUKE'S HOSPITAL

MEDICAL BULLETIN



VOL. 3 NO. 1

JANUARY 1964

Lymphangiography

Aldosterone Producing Tumor

Bone Implants

Urea and Urease Immunity
Review Article

Abstracts & Reviews

THE PRESBYTERIAN-ST. LUKE'S HOSPITAL MEDICAL BULLETIN

EDITOR

Steven G. Economou, M.D.

ASSOCIATE EDITORS

John P. Ayer, M.D.

Richard E. Buenger, M.D.

Joseph R. Christian, M.D.

Robert E. Dedmon, M.D.

Friedrich Deinhardt, M.D.

Charles V. Heck, M.D.

Philip N. Jones, M.D.

Sydney Kofman, M.D.

Max E. Rafelson, Jr., Ph.D.

Robert E. Slayton, M.D.

Milton Weinberg, Jr., M.D.

EDITORIAL ASSISTANT

Ann M. Ahtelius

HOSPITAL DIRECTOR

Norman A. Brady

The Presbyterian-St. Luke's Hospital Medical Bulletin is published quarterly by the staff of the Presbyterian-St. Luke's Hospital and its Alumni Foundation. It will be sent to those interested on request.

Address communications:

Editor

Presbyterian-St. Luke's Hospital Medical
Bulletin

1753 W. Congress Parkway

Chicago 12, Illinois

TABLE OF CONTENTS

<i>page</i>	
2.....	Lymphangiography FRANKLIN S. ALCORN, M.D. AND FRANK L. HUSSEY, JR., M.D.
16.....	Aldosterone Secreting Tumor of the Adrenal Gland: An Electron Microscopic Study HECTOR A. BATTIFORA, M.D.
24.....	Comparative Study of Bone Implants WILLIAM F. HEJNA, M.D. AND ROBERT D. RAY, Ph.D., M.D.
33.....	Urea and Urease Immunity ANDREW THOMSON, M.D.

LYMPHANGIOGRAPHY

FRANKLIN S. ALCORN, M.D.

FRANK L. HUSSEY, JR., M.D.

During the past two or three years, there has been increasing interest in lymphangiography as a method of demonstrating abnormalities of the lymphatic channels and lymph nodes. This interest is manifest by the increasing number of papers, post-graduate courses, and scientific exhibits concerned with this subject.

The purpose of this paper is to discuss the procedure used in the performance of these studies, and to consider briefly the method employed in most institutions¹. An intracutaneous injection of patent blue violet, (Alpha-Zurine 2-G) an aniline blue dye, is made distal to the lymphatic one desires to cannulate. The lymphatics of the skin and subcutaneous tissues selectively absorb this material, and are colored blue. A simple cut-down is made over a given lymphatic. This lymphatic is then cannulated with a fine needle attached to a polyethylene catheter. Ethiodol is then forced through the catheter into the lymphatic system by means of a mechanical pump. Ethiodol is an ethyl-alcohol ester of poppyseed oil, and contains 37% iodine.

Following cannulization and injection of a lymphatic in the lower extremity, the nodes in the inguinal, external iliac, common iliac and periaortic areas are roentgenographically visualized. Figs. 1, 2 and 3. The cisterna chyli and thoracic duct may also be seen. Fig. 4. When the injection is made in a lymphatic channel of the hand, the axillary and supraclavicular nodes are demonstrated.

The more common indications for lymphangiography are:

1. The differential diagnosis of intra-abdominal and pelvic masses.
2. The nature and extent of inflammatory and neoplastic disease which involves the lymphatic system.
3. The evaluation of edema of the extremities.
4. As a guide to lymph node dissections; particularly, when Ethiodol colored with chlorophyll is used.
5. The determination of abnormalities of the thoracic duct.
6. Evaluation of surgery, chemotherapy, and radiation therapy in malignant disease.

The difficulty in diagnosing intra-abdominal and pelvic masses by conventional X-ray methods is well known. Arteriography and venography are recent and useful additions to the time-honored studies of the gastrointestinal and genitourinary tracts. The recent development of lymphangiography, however, has made it possible to visualize the retroperitoneal, pelvic and abdominal lymph nodes, and has proven quite useful. It must be mentioned that all of the lymph nodes in the abdomen and pelvis are not shown. The inguinal, external, and common iliac, and periaortic nodes are shown well. The hypogastric chain and deeper nodes in the pelvis are only occasionally visualized. The lymph nodes adjacent to the renal hilus do not fill nor do those closest to the uterine cervix. In spite of this, it

From The Division of Radiology and Nuclear Medicine.



Fig. 1.—Normal peripheral lymph channels and lymph nodes during injection. a. Afferent channel; b. Femoral node; c. Efferent channel.



Fig. 2.—Normal pelvic lymph channels and nodes during injection.



Fig. 3.—Pelvic and abdominal lymph nodes 24 hours after injection. Note that nodes alone show and channels are empty.

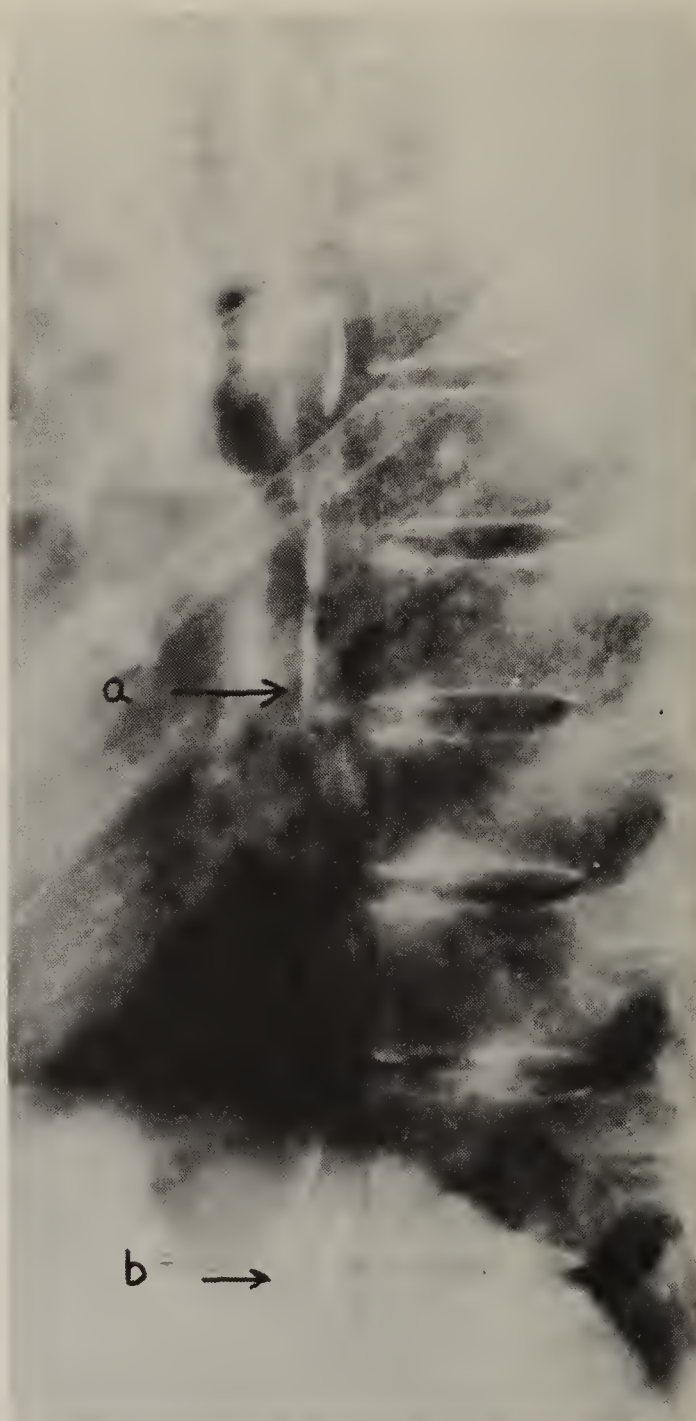


Fig. 4.—Lateral view of thoracic spine showing thoracic duct. It is seen only on day of injection. a) Thoracic duct; b) Area of cisterna chyli.

has been found that lymphangiography is the most sensitive diagnostic method for evaluating the size of the lymph nodes in the pelvis, as well as in the retroperitoneal space about the aorta. In a series of 85 patients who were studied sequentially by lymphangiography, intravenous urography and inferior vena-cavagraphy, positive lymphograms were found in 65% of the patients with proven malignant disease². There are also a few cases where obscure abdominal masses have been proven to be a group of enlarged lymphomatous nodes by lymphography, after other diagnostic methods had failed. (Figs. 5 and 6).



Fig. 5.—Lymphangiogram on undiagnosed abdominal mass reveals extensive lymphoma. a. Generalized enlargement with diffuse reticular pattern.

It is in the detection and evaluation of the nature and extent of neoplastic and inflammatory diseases involving the lymphatic system, that the study finds its principal use. It is of greatest value in the management of patients with neoplastic disease, especially malignant lymphoma. The reason for the high

degree of accuracy in lymphography of the lymphomas is the diffuse lymph node involvement with enlargement and abnormal architecture, which is characteristic and which is usually visualized on the roentgenograph. In lymphoma, we have seen the following adenographic patterns:



Fig. 6.—Same patient as in Fig. 5 following radiation therapy to pelvic and abdominal nodes. Note marked diminution in size of lymph nodes.

1. Generalized enlargement with diffuse reticular pattern. (Fig. 5).
2. Marked increase in the number of the nodes clustered about a certain area. (Fig. 7).
3. Nodal enlargement with scattered, irregular, filling defects. (Fig. 8).

These patterns are often seen together in the same patient, as shown in Figs. 6, 7 and 8. Some authors feel that it is possible to differentiate one type of

lymphoma from another, by means of lymphangiography. However, we do not feel that this can be done with a great degree of accuracy. Many agree that cases of lymphosarcoma are occasionally encountered which will resemble metastatic carcinoma³. Involvement of abdominal nodes in sarcoidosis and tuberculosis mimic lymphoma very closely.

In diagnosing nodal involvement, in metastatic carcinoma, the accuracy is



Fig. 7.—Marked increase in the number of nodes clustered about a certain area. a. Many nodes in presacral area.



Fig. 8.—Enlarged pelvic nodes showing multiple radiolucent filling defects. a. Radiolucent defect.

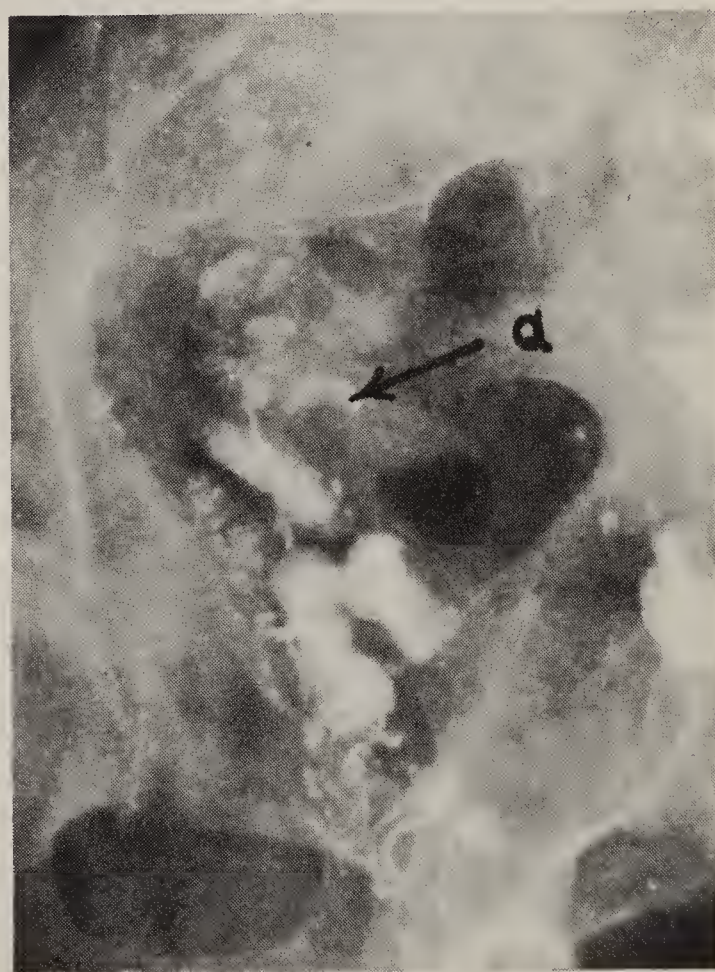


Fig. 9.—Patient with known urinary bladder carcinoma. a. Lymph node with radiolucency representing metastatic disease.



Fig. 10.—Normal femoral nodes and channels. Note how channels cluster near hilus of nodes.

not as high as in the lymphoma group. Although metastatic carcinoma can be demonstrated in the lymph nodes on many occasions, the value of lymphography in the study of patients who have early primary lesions, and no other evidence of tumor spread, has not yet been established⁴. It is difficult to demonstrate metastases if they are small, and impossible if microscopic. Metastases must be at least 4mm in diameter before they may be identified on the lymphogram⁵. (Fig. 9). Nodes with areas of fat necrosis or fibrosis as well as nodes that are incompletely filled with contrast material, often mimic metastatic nodes. There are often radiolucencies at the hilus of a node which mimic metastatic

carcinoma, a finding most often seen in the femoral region. (Figs. 10 and 11). Frequently, a node can be completely replaced by carcinoma. In such an instance, the diagnosis cannot be made by noting its absence since the number and location of nodes in a given group are inconstant. When there are multiple radiolucencies in a given chain of nodes, which may or may not be enlarged, and there are displaced channels and multiple collaterals caused by obstruction, the diagnosis of metastatic carcinoma is easier. (Fig. 12). Unfortunately, by this time, the primary lesion is generally far advanced, and the spread is strongly suspected and often readily diagnosed by other means.

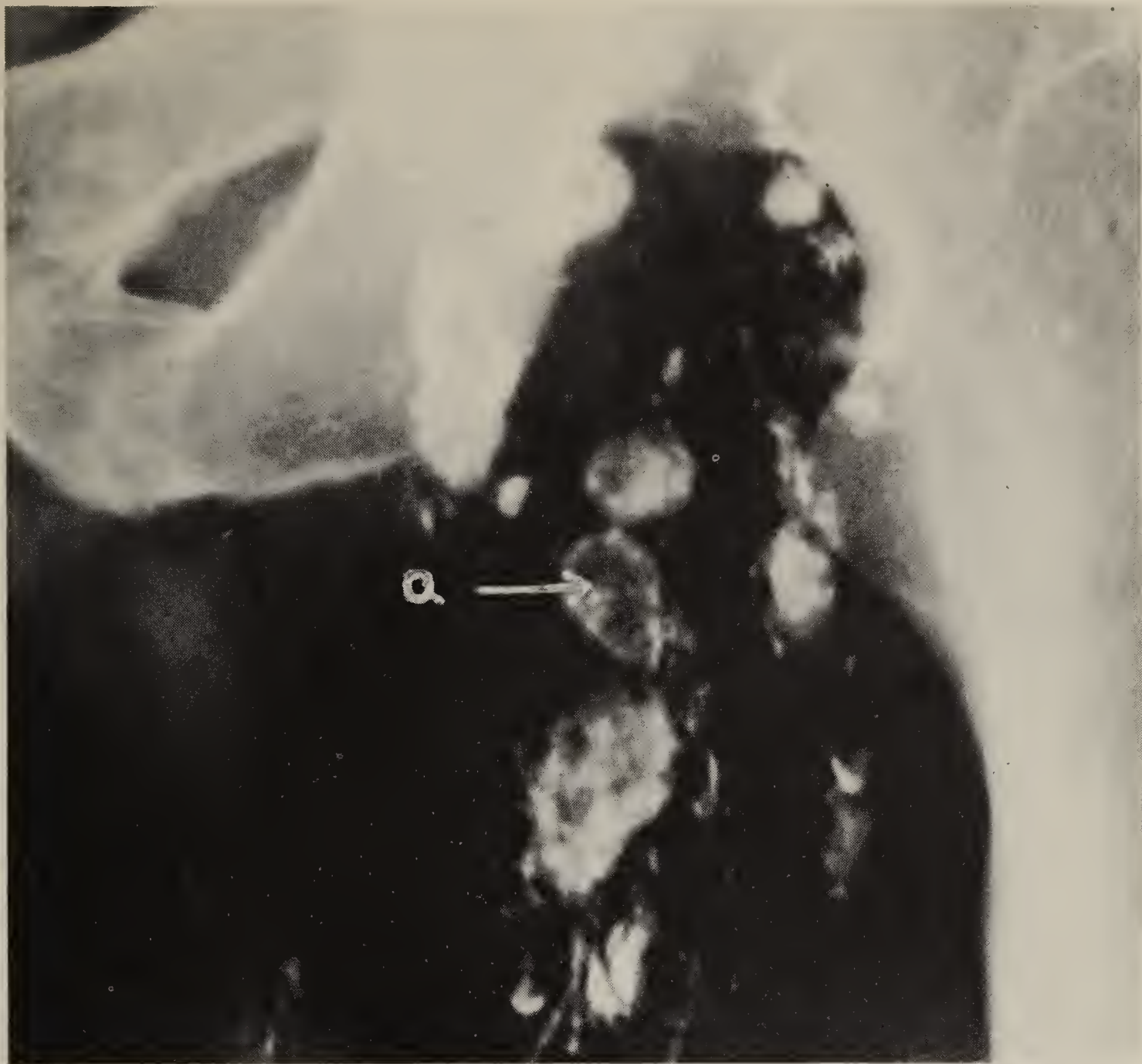


Fig. 11.—Normal femoral nodes the day after injection. a. Radiolucency at hilum seen when channels are empty.

Until the advent of this new method of study the status of the lymphatics in patients with primary lymphedema had remained obscure. Kinmonth in 1954, described his technique for examination of the lymphatics of the lower extremity⁶, and again in 1957, presented his findings in patients with primary lymphedema of the lower extremities⁷. Lymphedema is generally divided into two types: the first is primary, or so-called, non-inflammatory, and the second, is acquired or obstructive lymphedema. In the primary lymphedemas, which usually arise before the age of 35 years or in the familial congenital type (Milroy's Disease), studies show that the lymph

vessels are malformed. The range of abnormality extends from aplasia and hypoplasia of the lymph channels, to numerous but dilated, or varicose channels. Most patients seem to have hypoplasia of the lymph channels, and they frequently have only one channel passing from the foot to the groin, as opposed to five or six that are normally seen. These patients usually do fairly well until early adult life, when unexplained lymphedema arises. Occasionally, this is preceded by an episode of inflammation or cellulitis, which might cause transient edema in a normal subject, but results in permanent edema in these patients. More often there is no clinically

significant precipitating episode. In the opinion of Kinmonth, the abnormal vessels are sufficient to maintain drainage of tissue fluid under ordinary circumstances. Should the amount of lymph be temporarily increased by the formation of inflammatory exudate, or other cause, patients with primary lymphedema are apparently unable to drain off this excess. An interesting abnormal lymphographic finding seen in some cases of

primary lymphedema, is described as, "dermal backflow." Here, there are multiple patches of dilated lymph vessels in the skin. These vessels communicate with the deep channels, and much of the fluid is entrapped therein. (Fig. 13).

Lymphography in the acquired post-surgical edema, following mastectomy, demonstrates the expected. There is an absence of the channels extending to the axillary area, and beyond, therefore,

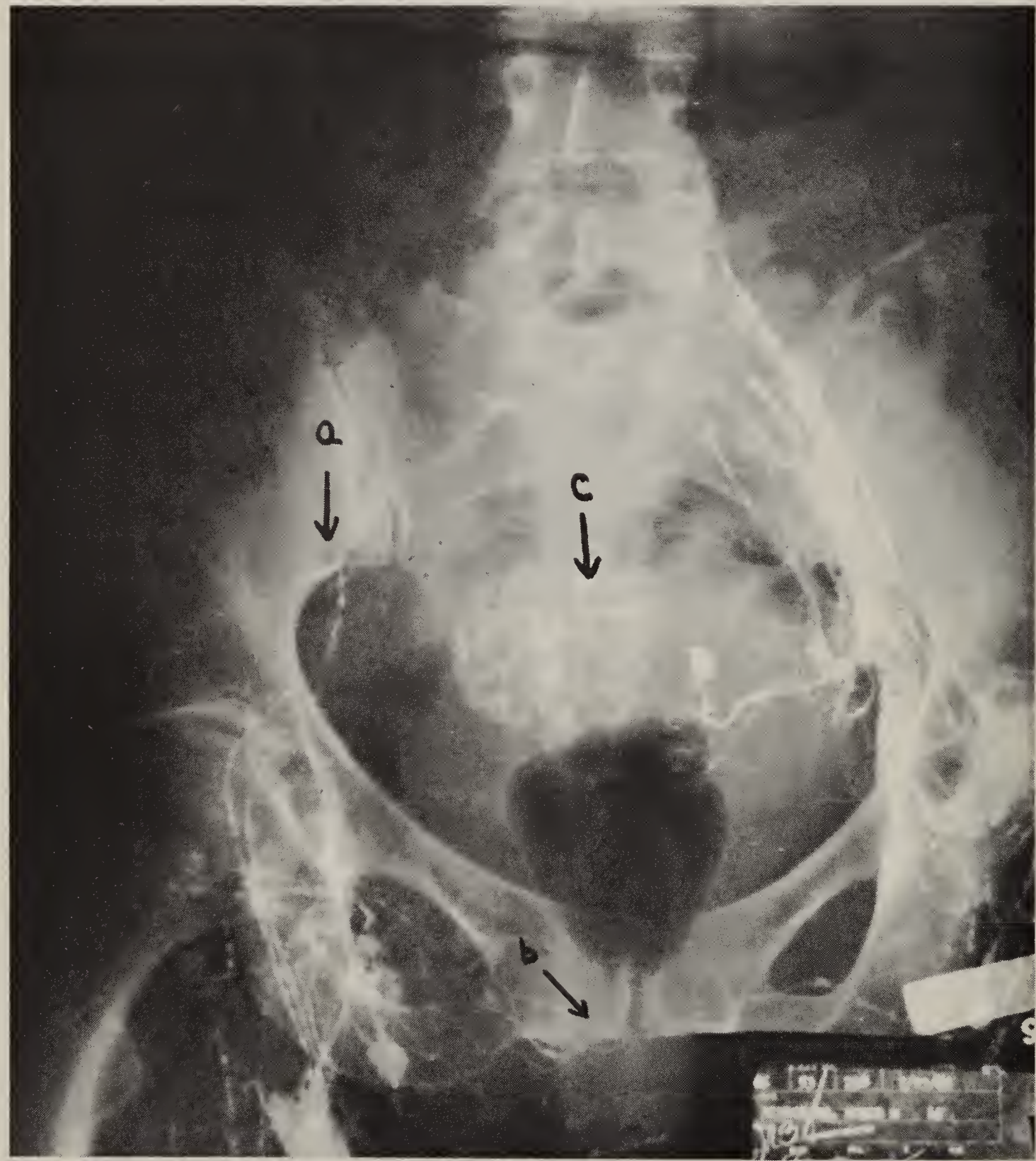


Fig. 12.—Patient with previously treated stage III carcinoma of the cervix who developed unilateral edema of the right lower extremity. a. Area of blocked lymph nodes in right pelvis. b. Many collateral channels in the skin carrying contrast material across the pelvis. c. Irregular calcifications in mid-pelvis, an unrelated uterine fibroid.

the lymphatic channels of the skin carry the major load of fluid toward the trunk. The overfilling of these channels causes obvious lymphedema. (Fig. 14).

An additional use of lymphography lies in the demonstration of a given group of lymph nodes prior to surgical resection. Generally, Ethiodol, colored with chlorophyll is used, so that the lymph nodes will be stained green. This facilitates considerably their identification and subsequent surgical removal. After the dissection and before closure of the operative wound a film taken on the operating table will reveal the thoroughness of the procedure. If remaining lymph nodes are seen on the roentgenogram, further dissection may be undertaken which can be checked a second or even a third time, by film examinations. (Fig. 15 and Fig. 16).

Finally, an evaluation of chemotherapy and radiation therapy may be assisted by the use of appropriate lymphographic studies. Decrease in the size of the lymph nodes, following chemotherapy or radiation therapy, may be demonstrated easily. (Figs. 5 and 6). We have found that in patients with malignant lymphoma in remission, there is occasionally a genuine diminution in size and number of nodes. (Fig. 17). When there is a return of clinical symptoms, occasionally re-enlargement is seen of the nodes previously reduced by the therapy. Another advantage in radiation therapy, is the accuracy with which X-ray therapy portals may be placed over involved lymph nodes. (Fig. 18).

The administration of chemotherapeutic and radioactive therapeutic substances, via the lymphatic route, has been reported^{8,9}. Many agents, both water soluble and oil-based have been tried. The aqueous solutions are not satisfactory, as there is considerable spill into the adjacent tissues, when the substance reaches the lymph nodes. Because they are water-soluble the chemo-therapeutic substances find their way into the blood stream quite rapidly and high blood levels of the chemical are reached so that the differential advantage in the use of



Fig. 13.—“Area of dermal backflow” in a case of primary lymphedema in a 13 year old girl.

the lymphatic route is lost. Lymph nodes which have been replaced by tumor will not admit the therapeutic agent, as there is no remaining normal lymphatic tissue. In spite of these difficulties, the method is still under investigation, and the development of a more suitable agent which

will permeate all of the lymph nodes may be found.

As in any new procedure, the value of information gained must be weighed against any possible injury to the patient. Aseptic technique is sufficient to prevent local wound infection, and subsequent



Fig. 14.—Post-mastectomy edema of right arm. a. Many dilated and tortuous lymphatics of the skin. b. Main channels are absent. c. Supra-clavicular and some axillary nodes remain.

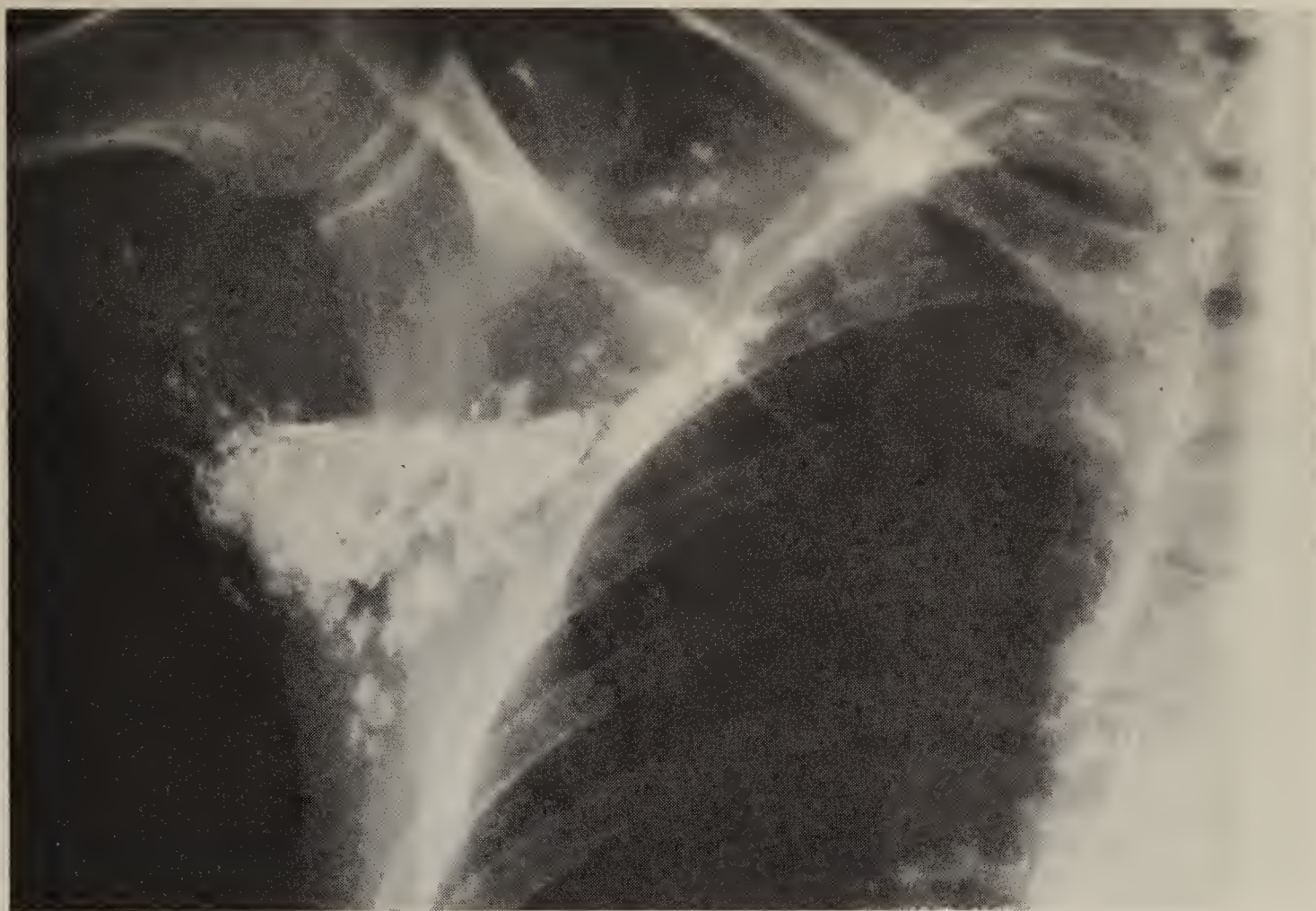


Fig. 15.—An alleged positive axillary lymph node in patient with melanoma. Chlorophyllated Ethiodol was used.

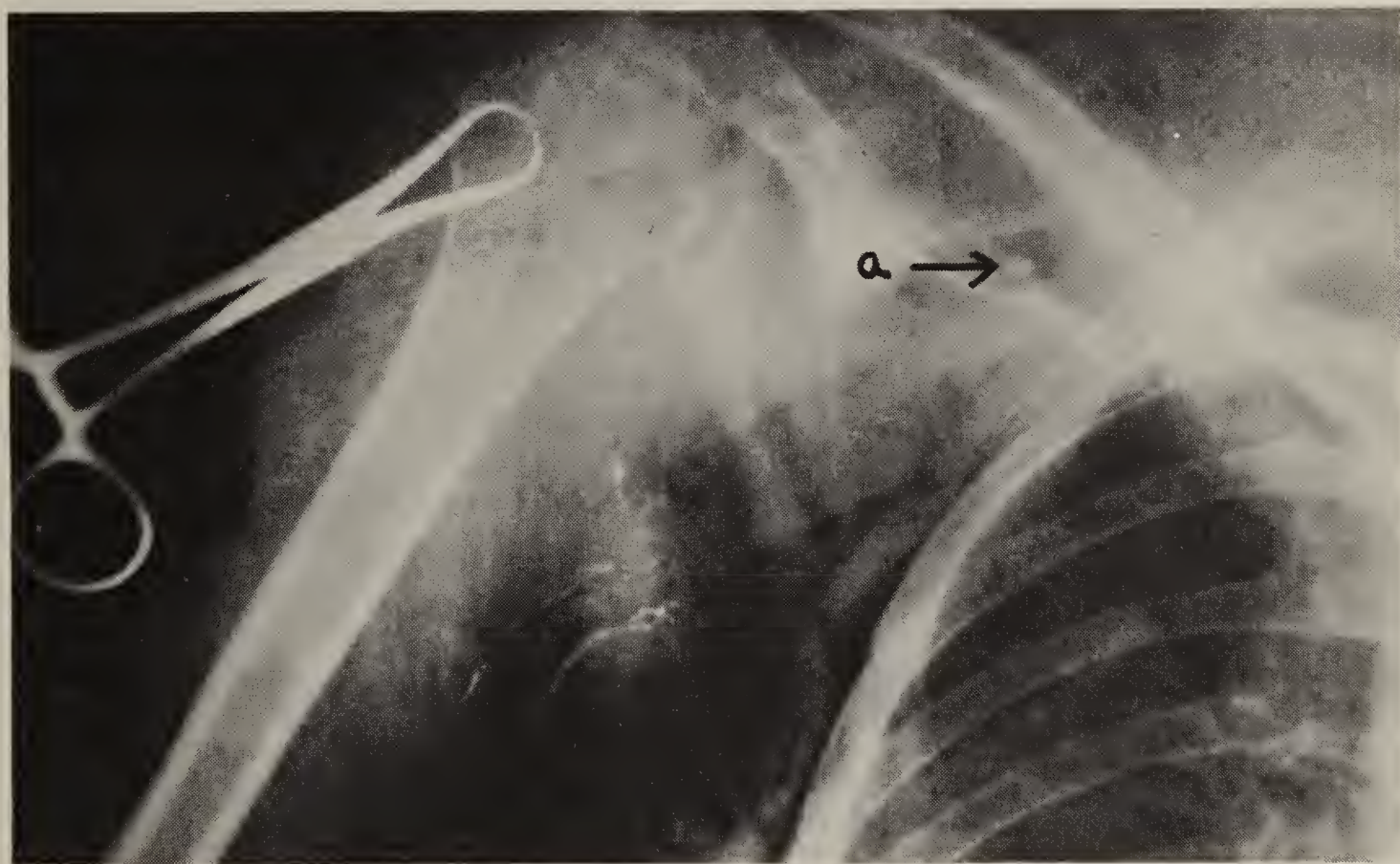


Fig. 16.—Portable film on operating table shows removal of nodes. (Same patient as Fig. 15). a. 1 small remaining node which was then removed.

ascending lymphangitis. It has been shown that oil is deposited in the lungs and to a lesser extent, in the reticulo-endothelial system of the liver, spleen and kidneys⁵. There has been no proof of profound or long-lasting damage to any of these organs. The patients occasionally develop temperatures in the



Fig. 17.—Lateral view of lumbar spine showing para-aortic lymph chains and a solitary node.



Fig. 18.—Lymphomatous femoral node—lead strips outline the treatment portal.

range of 100°F to 102°F, on the day of the procedure. This is felt to be the result of a foreign body reaction of oil droplets in the lungs. These affects are usually transitory, and respond quickly to antipyretics. In one of our cases, a very substantial amount of oil was injected inadvertently into the veins, and thence flowed into the vessels of the lungs. This patient developed a very high fever for several days, accompanied by some dyspnea, but responded rapidly to antipyretics and cortisone. There were no obvious sequelae and an uneventful recovery was made.

The question is always raised concerning the possibility of dislodgment of malignant cells from lymph nodes and their subsequent spread throughout the body during lymphangiography. Studies have been done to determine the increase

in circulating tumor cells in the blood during and following lymphangiography^{5,10}. Thus far, there has been no conclusive evidence that a significant number of cells are displaced by the procedure.

CONCLUSIONS

Lymphography has its greatest value in the management of patients with malignant lymphoma. It is of less value in patients with carcinoma. In patients with carcinoma, however, it has been useful as an aid in identification of lymph nodes during surgery, and as a means of checking the completeness of the surgical dissection.

The uses of lymphangiography are currently being investigated by a large number of workers. Should the reliability of the findings persist, it will then be incorporated into the host of valuable and frequently used X-ray procedures.



REFERENCES

1. Wallace S. et al.: Lymphangiograms: Their Diagnostic and Therapeutic Potential; *Radiol.* 76:179, Feb., 1961.
2. Stanley Baum, et al.: Lymphangiography, Inferior Venacavagraphy, and Intravenous Urography—Comparative Accuracy in the Diagnosis of Pelvic and Abdominal Masses. *Radiology* 81:207-218, August, 1963.
3. Manuel Viamonte, et al.: Radiographic-Pathologic Correlation in the Interpretation of Lymphangiogram. *Radiology* 80:903-916, June, 1963.
4. Lymphography—An Editorial, H. W. Fischer, *Radiol.* 80:1002, June, 1963.
5. Burton Schaffer et al.: A Critical Evaluation of Lymphangiography. *Radiology* 80:917-930, June, 1963.
6. Kinmonth, J. B.: *Ann. R. Coll. Surg. Eng.*, 15:300, 1954.
7. Kinmonth, J. B.: Primary Lymphedemas. *British J. Surg.* XLV:1-9, July, 1957.
8. Ariel, Irving: The Use of Radioactive Isotope in the Treatment of Lymphomas. *Am. J. Roentgen.* 90:311-323, August, 1963.
9. Jantet, G. H.: Direct Intralymphatic Injection of Radioactive Gold in the Treatment of Malignant Diseases. *Brit. J. Radiology* 35:692-697, Oct., 1962.
10. Roberts, et al.: Technique and Results of Isolation of Cancer Cells From the Circulatory Blood. *Arch. Surg.* 76:334-346, March, 1958.

ALDOSTERONE SECRETING TUMOR OF THE ADRENAL GLAND: An Electron Microscopic Study

HECTOR A. BATTIFORA, M.D.

INTRODUCTION

The ultrastructure of the adrenal cortex has been studied in several species 1,2,3 as well as man 4,5. To the best of our knowledge there have been no reports of aldosterone-secreting tumors studied with the electron microscope. The present paper deals with the ultrastructural aspects of an adrenal cortical tumor obtained from a 53 year old woman with primary aldosteronism treated with right adrenalectomy.

GROSS AND MICROSCOPIC PATHOLOGY

The adrenal contained a yellow cortical adenoma measuring 2 cm. in diameter. The cortex appeared somewhat thickened. With the light microscope, the tumor was composed of cords and groups of cells resembling those of normal zona glomerulosa. The tumor was poorly circumscribed. Throughout the adrenal cortex, small adenomata of similar appearance were present.

ELECTRON MICROSCOPY

Immediately after its removal, a representative sample of the tumor was minced into 1 sq. mm. blocks with a sharp razor blade and fixed in chilled osmium tetroxide, buffered to pH 7.4. After a two hour fixation, the blocks were dehydrated in graded series of alcohol and embedded in Epon-epoxy resin. Sections, 0.5 to 1 micron in thickness, were made with a Porter-Blum microtome and, after selection of the proper area, ultrathin sections were cut with an LKB ultramicrotome equipped with a diamond knife. The sections were mounted in formvar-carbon coated copper grids and studied in an RCA EMU 3E electron microscope.

From the Division of Pathology

With the electron microscope, the cells were of uniform appearance and no "dark" and "clear" cells, as described in the adrenal cortex by some investigators were found. They had round centrally-located nuclei with finely-clumped marginated chromatin but small masses of chromatin were also disseminated throughout the karyoplasm. A double nuclear membrane with nuclear pores was often encountered. The cytoplasm contained the typical lipid vacuoles described by investigators of adrenal cortex ultrastructure. (Fig. 1). Some vacuoles had a peripheral electron-dense halo but the majority were empty-appearing and bounded by single membranes. A few dense lipid droplets were also seen within the cytoplasm of the epithelial cells. Large numbers of microvacuoles, possibly related to smooth-surfaced endoplasmic reticulum, were found as well as free ribosomes occasionally forming clusters but not associated with membranes. The Golgi apparatus was inconspicuous. The mitochondria were large, round, or bean-shaped and predominantly of the tubulosaccular variety, although some had membranous cristae and occasional ones were of mixed type. Frequently the mitochondria were deformed by the pressure of the adjacent lipid vacuoles. No transi-

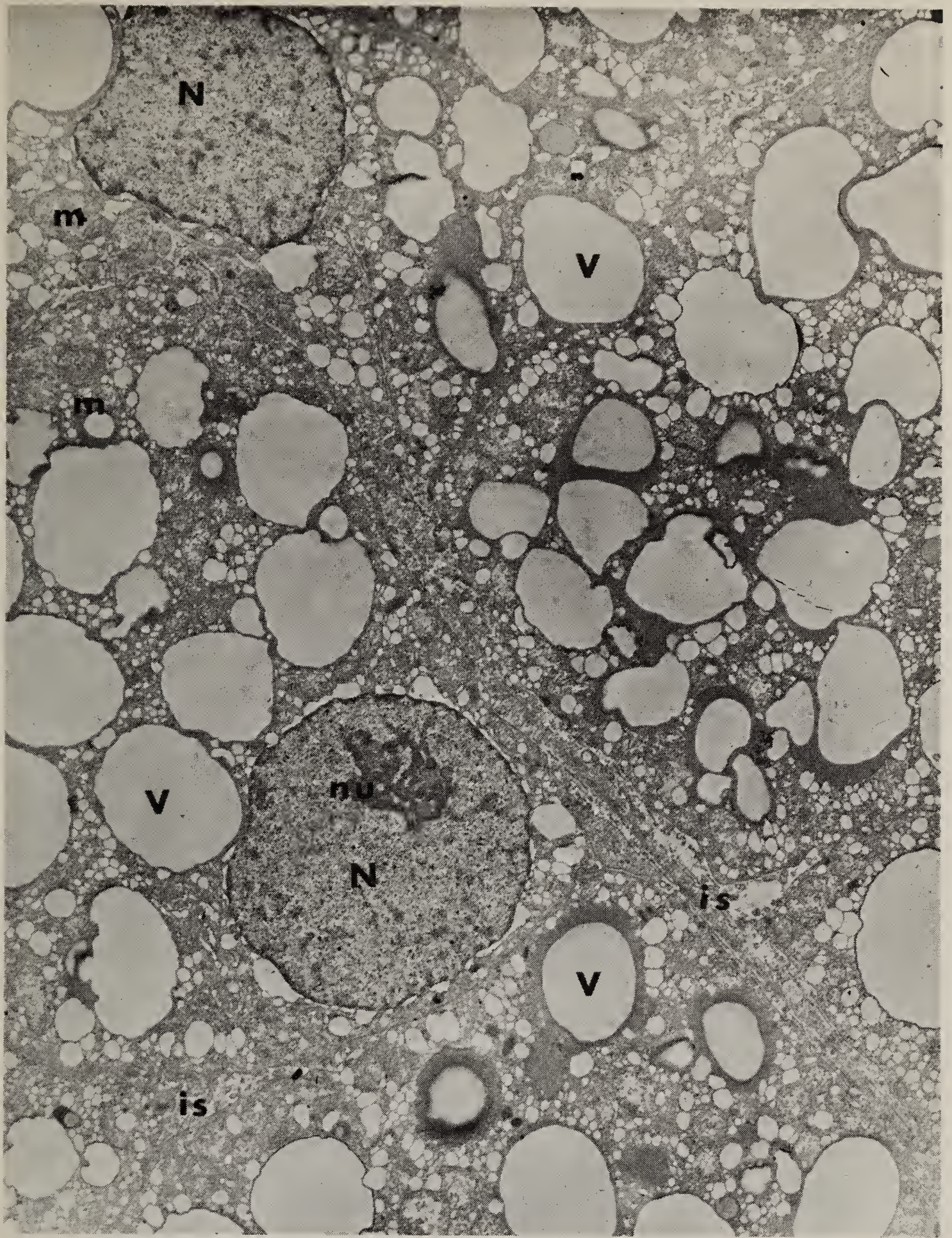


Fig. 1.—Scanning electron-micrograph of the adrenal tumor. Large round vacuoles are seen. Some possess osmiophilic rims. (N) nucleus; (nu) nucleolus; (V) vacuole; (is) intercellular space; (m) mitochondria X3,500.

tional forms between mitochondria and lipid vacuoles were seen. (Fig. 2).

The cells were associated by few interdigitations and possessed no desmosomes. They were frequently separated by intercellular spaces containing microvilli. The intercellular space was often continuous with the peri-endothelial space. The latter was wide and sometimes contained prolongations of adjacent epithelial cells. Frequently it contained fragments of epithelial cells and collagen bundles. Unmyelinated nerve fibers were

found regularly in association with capillaries in the peri-endothelial space. (Fig. 2). Two basement membranes were present in this space. One corresponded to the epithelial cells and another to the capillary. Both membranes, especially the former, were discontinuous and club-shaped prolongations of epithelial cells were seen protruding into the peri-endothelial space through the fenestrations of the epithelial basement membrane. (Fig. 3). These peculiar structures were different from the frequent micro-

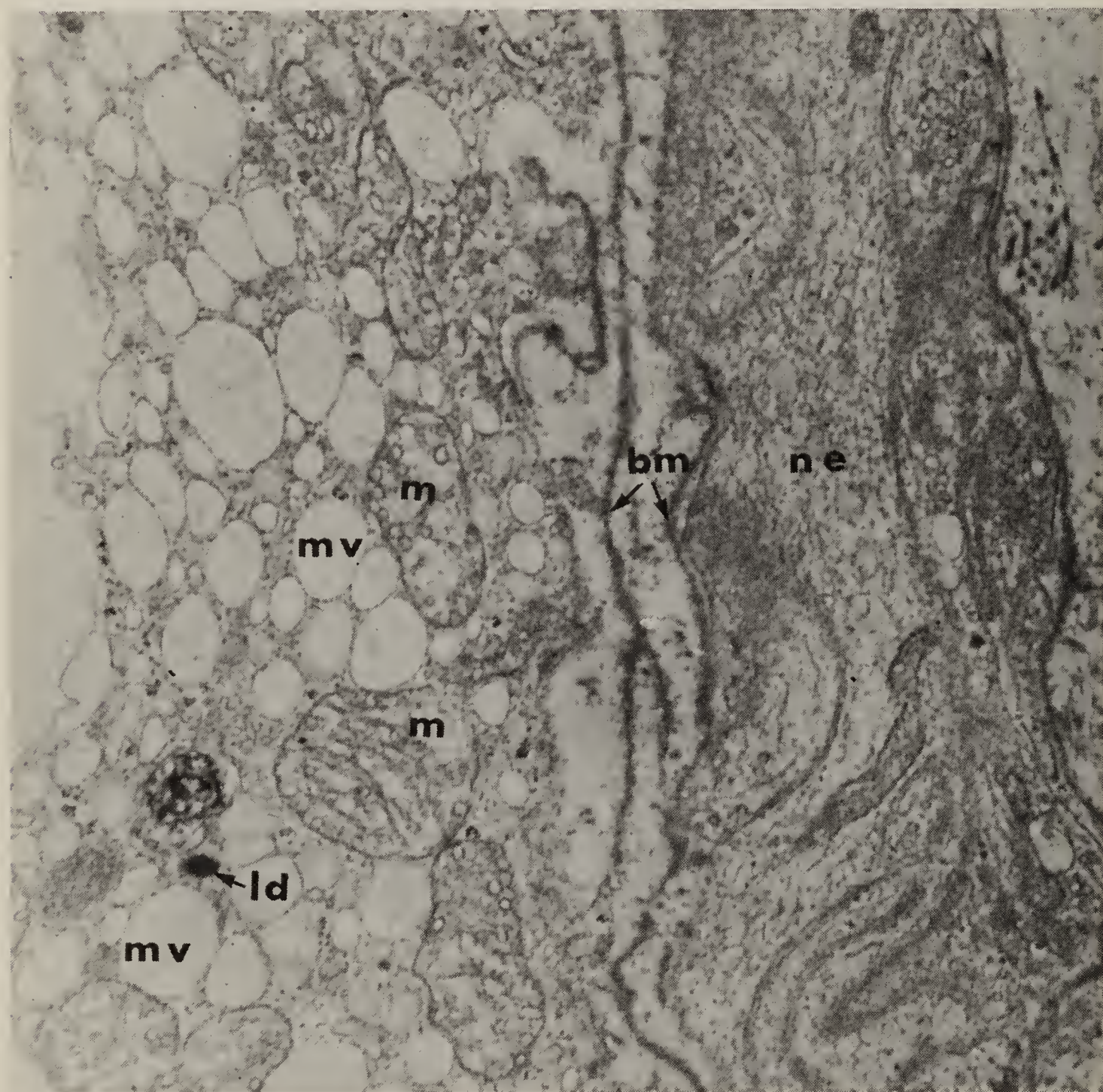


Fig. 2.—Portion of an epithelial cell and a nerve ending (ne). Tubulomembranous mitochondria (m), micro-vacuole (mv), (bm) basement membrane, and (l) lipid droplet. X24,300.

villi found between the epithelial basement membrane and the cell membrane, and often contained vacuoles. In addition to this finding, fragments of cells, isolated mitochondria and lipid-membrane complexes were seen within the widened periendothelial space. (Fig. 4). Indeed, large fragments of tumor cells and entire cells were occasionally seen filling the lumen of capillaries. Many capillaries were of the sinusoid type and,

in addition to fenestrated basement membranes, they exhibited true gaps in their endothelial cell cytoplasm. Fragments of tumor cells were, at times, seen traversing such openings. (Fig. 5). Many endothelial cells possessed cytoplasmic vacuoles and lipid droplets similar to those encountered in epithelial cells. The latter were, at times, enclosed in single-layered membranes.

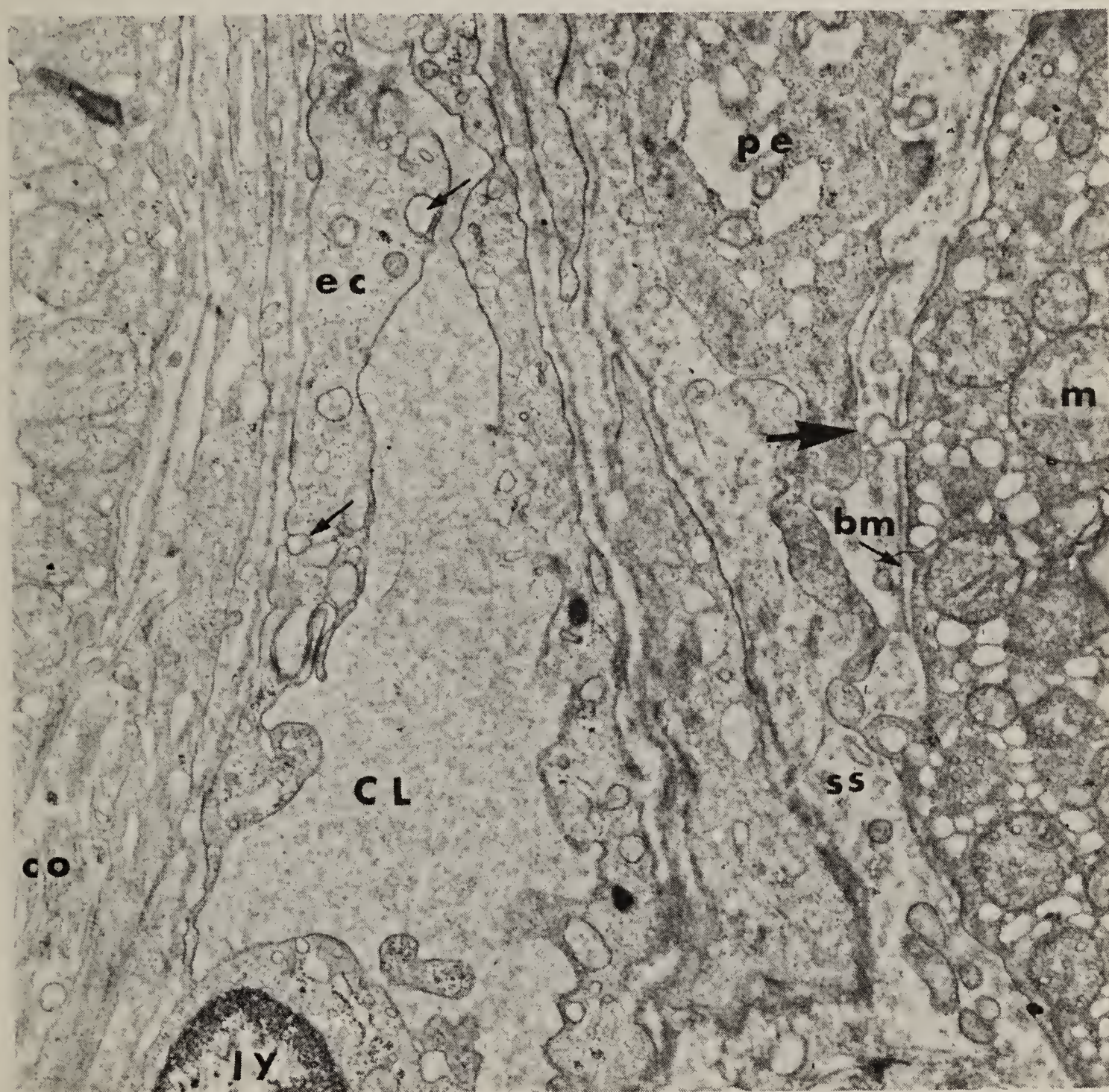


Fig. 3.—Capillary, subendothelial space (ss) and portion of tumor cell. Thick arrow points toward a vacuolated cytoplasmic prolongation protruding through a fenestration in the epithelial basement membrane (bm). (Cl) capillary lumen; (ly) lymphocyte; (ec) endothelial cell (thin arrows point toward pinocytic vesicles); (m) mitochondria; (pe) prolongation of pericyte's cytoplasm; (co) collagen. X12,100.

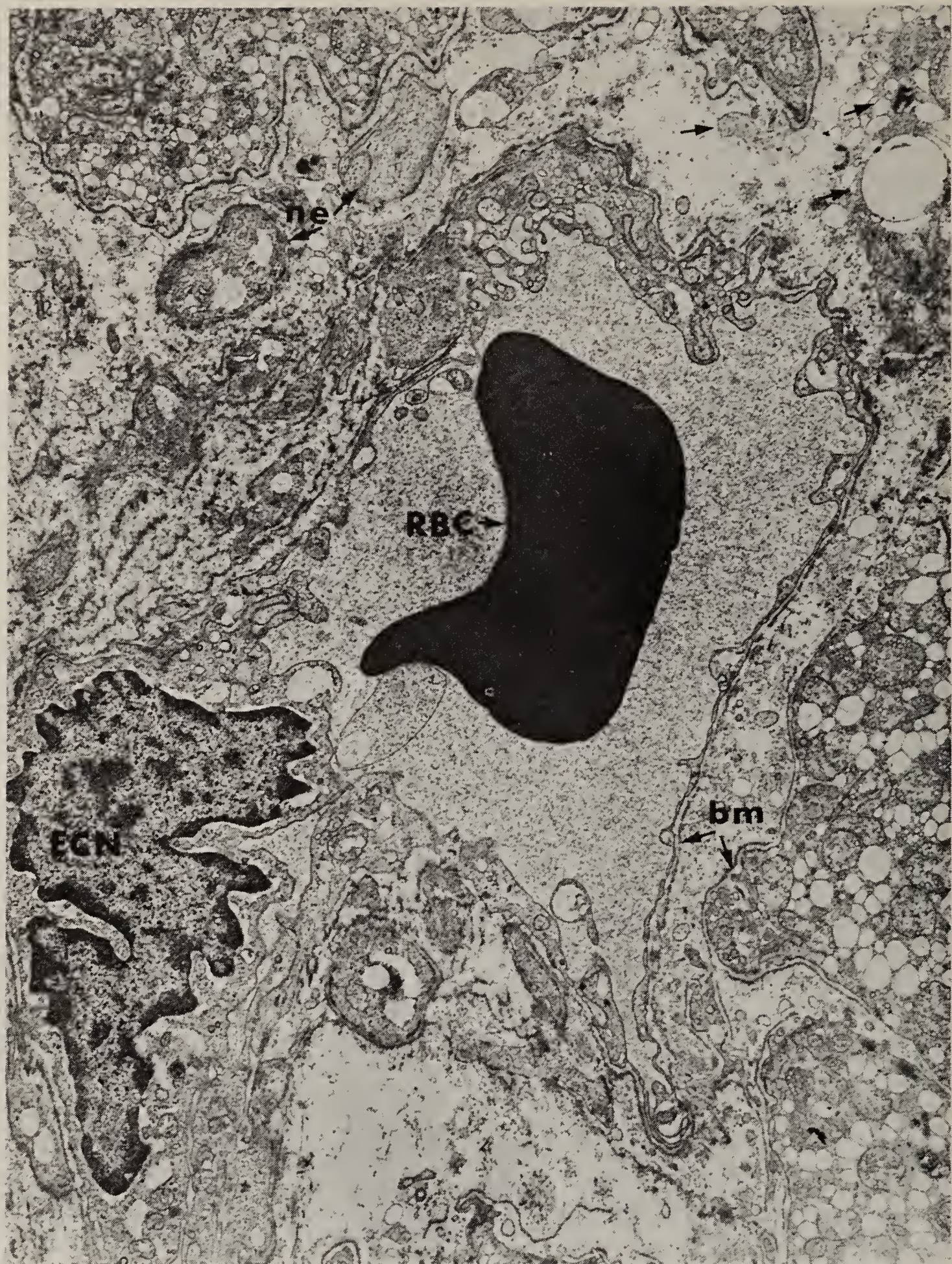


Fig. 4.—Capillary and periendothelial space containing fragments of cells (upper right hand, arrows). (RBC) red blood cell; (ECN) endothelial cell nucleus; (bm) basement membranes of capillary and epithelial cell; (ne) nerve endings. X7,850.

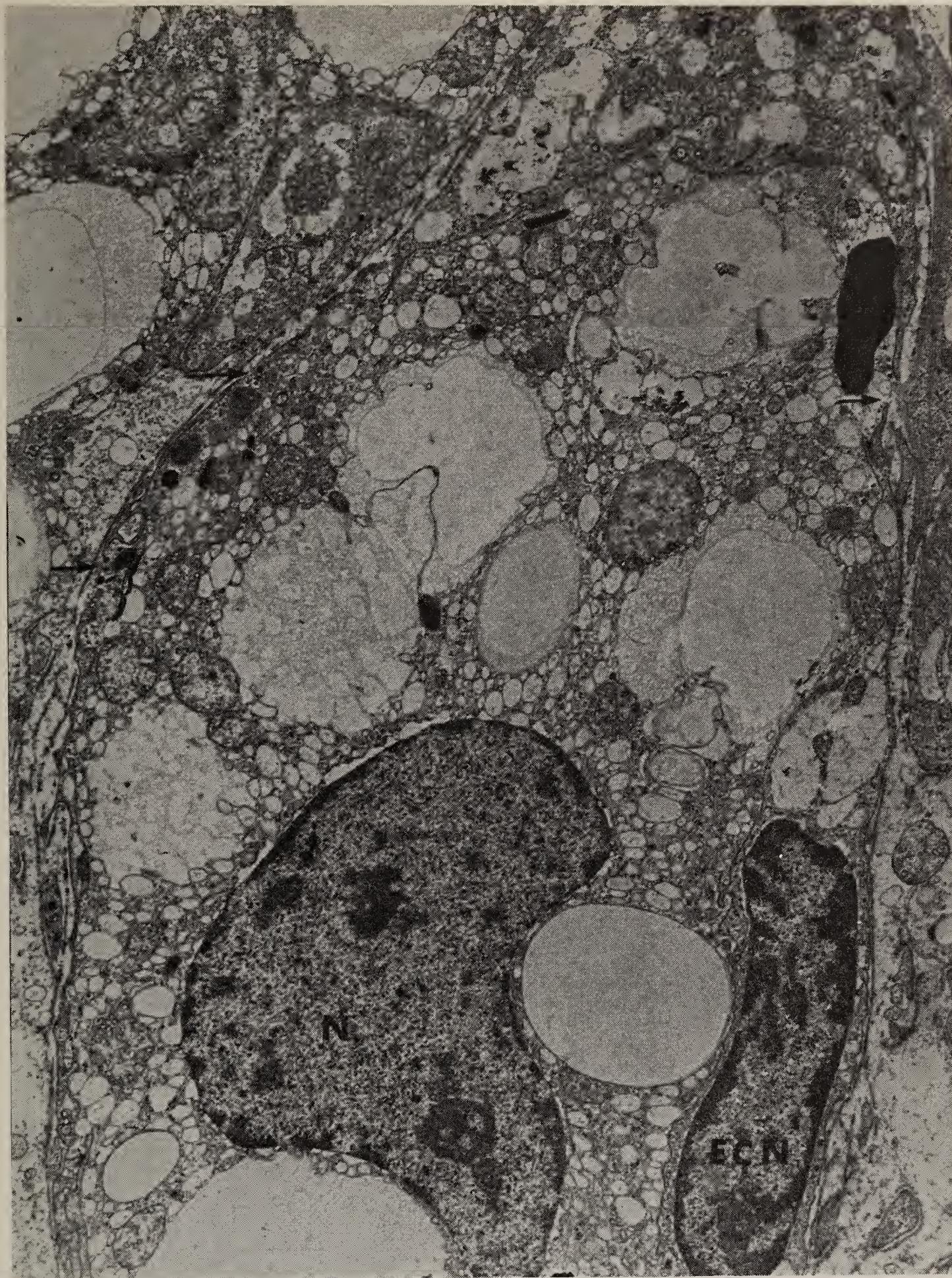


Fig. 5.—Sinusoidal capillary containing a well-preserved tumor cell. Arrows point to gaps in the endothelial lining. (ECN) endothelial cell nucleus; (N) tumor cell nucleus. X8,100.

DISCUSSION

The ultrastructure of this tumor resembles that of normal zona glomerulosa of adrenal cortex. The absence of "clear" and "dark" cells is in keeping with Lever's idea 1,6 that they merely represent different stages in cyclical secretory activity of the same kind of cell and may indicate that in the tumor all cells were in a similar secretory period.

We could not find transitional images between mitochondria and lipid vacuoles although some mitochondria contained lipid inclusions. Lever revived the concept that the lipid vacuoles resulted from transformation of mitochondria but this idea has been rejected by most authors.

Two possible types of release of hormonal products can be visualized in this

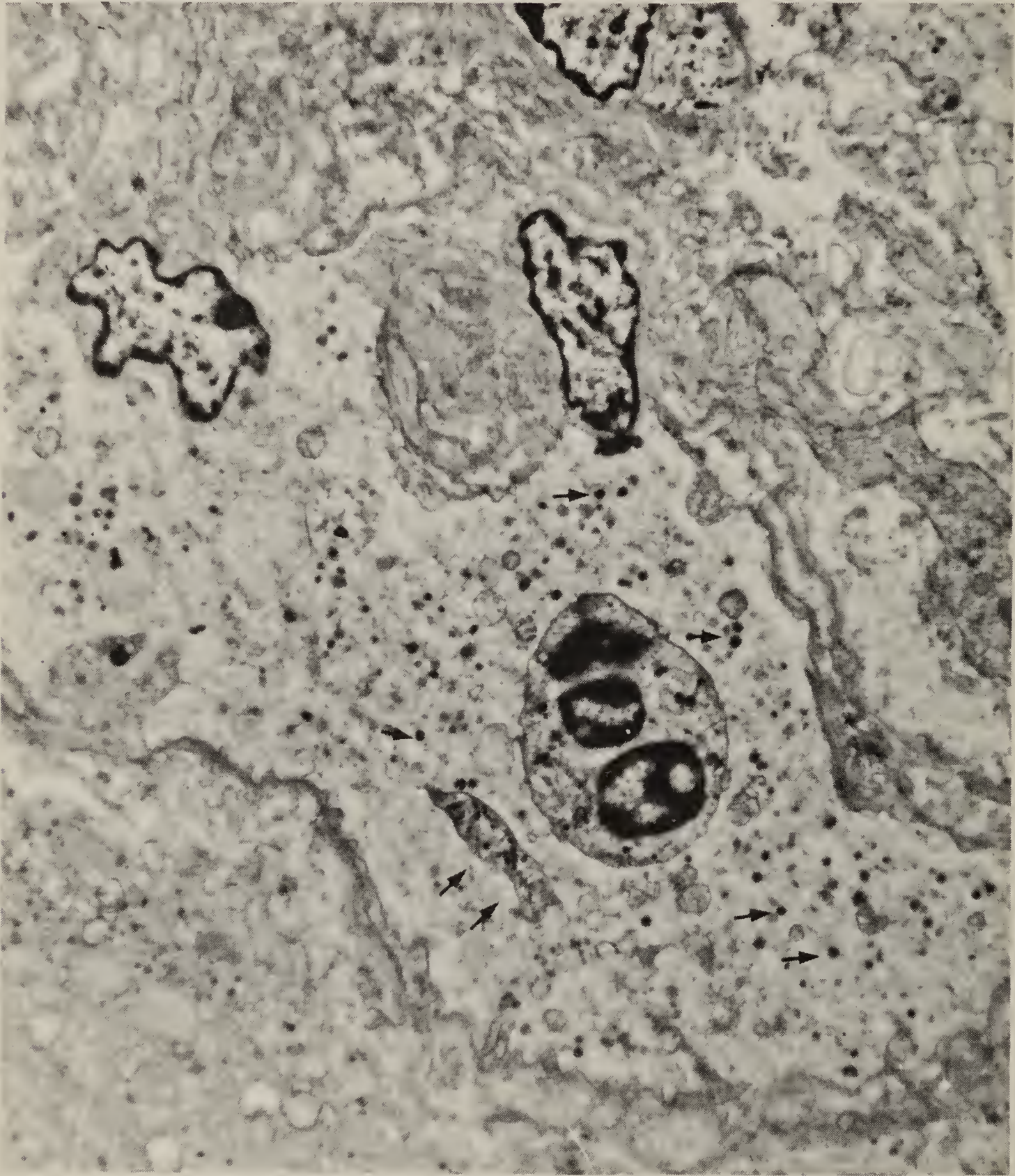


Fig. 6.—Capillary in pheochromocytoma containing a leukocyte, cellular fragments and numerous secretory granules (arrows) presumably representing hormonal packets. Similar granules are seen in the cytoplasm of tumor cells in the upper right hand and lower left hand corners. Mag. approx. X6,000.

neoplasm. In one, submicroscopic fragments of cells are released through the perforations in the epithelial basement membrane into the subendothelial space. In the other, large fragments of cells, through interruptions of the cellular membrane, gain access into the subendothelial space and from there into the sinusoids. Indeed, well-preserved mitochondria and large cell fragments were seen with blood vessels. Cotte and Cotte 7 found similar images in normal hamster adrenal cortex and this favors the view that this represents holocrine glandular activity rather than artefact

induced by handling of the tissue. However, the mechanical process of handling the organ may enhance this naturally occurring phenomenon which, incidentally, is not privative of the adrenal cortex and has been observed by Eisenstein 8 in a pheochromocytoma (Fig. 6), giving morphological confirmation to the fact that hypertensive bouts can be precipitated by abdominal massage or handling of the tumor. An interesting finding was the presence of numerous nerve endings suggesting that the tumor might perhaps respond to normal neurologic stimuli for hormonal release.

SUMMARY

The ultrastructure of an aldosterone-secreting adrenal cortical adenoma is described and illustrated. The cells resemble those of the normal zona glomerulosa. Based on morphological evi-

dence, two possible mechanisms of release of hormonal products are suggested. The presence of terminal nerve fibres amidst tumor cells is noted and briefly commented upon.

ACKNOWLEDGMENT

The author is grateful to Drs. P. Winter and V. Pollack who supplied him with the specimen, to Drs. George M. Hass and Reuben Eisenstein for their assistance in preparing the manuscript and to Mr. Leonas Zeruolis for his technical help.

REFERENCES

1. Lever, J. D.: Electron Microscopic Observations on the Adrenal Cortex. *Am. J. Anat.*, 97:409-430, 1955.
2. Fujita, H.: An Electron Microscopic Study of the Adrenal Cortical Tissue of the Domestic Fowl. *Z. Zellforsch.*, 55:80-88, 1961.
3. Sabatini, D. D., and DeRobertis, E.: Ultrastructural Zonation of Adrenocortex in the Rat. *J. Biophys. Biochem. Cytol.*, 9:105-119, 1961.
4. Carr, I. A.: Microvilli of the Cells of the Human Adrenal Cortex. *Nature*, 182:607-608, 1958.
5. Ross, M. H.: Electron Microscopy of the Human Foetal Adrenal Cortex. In *The Human Adrenal Cortex*. (A. R. Currie, T. Symington and S. K. Grand, eds.)
6. Lever, J. D.: Electron Microscopic Observations on the Normal and Denervated Adrenal Medulla of the Rat. *Endocrinology*, 57:621-635, 1955.
7. Cotte, G., and Cotte, M.: Etude Ultrastructurale D'Images De Fonte Holocrine Dans La Cortico-Surrenale. *Z. Zellforsch.* 54:182-198, 1961.
8. Eisenstein, R. Personal Communication.

COMPARATIVE STUDY OF BONE IMPLANTS

WILLIAM F. HEJNA, M.D.

ROBERT D. RAY, PH.D., M.D.

INTRODUCTION

The purpose of the following study was to compare histologically and roentgenographically, the replacement of materials placed in defects in the radii of rats. These materials included demineralized bone, Plaster of Paris, collagen, and fresh autogenous bone. The project represents a continuing effort to find a satisfactory substitute for fresh autogenous bone which would be readily available and easily prepared and stored. Since the days of Barth and Auxhausen, most investigators have agreed that autogenous bone is superior to other materials on the basis of cell survival, host acceptance, vascularization, resorption, and rate of replacement¹⁻⁹. On the other hand, the use of fresh autogenous bone grafts usually requires additional surgery and additional operating time, and even with autogenous grafts, there is a significant incidence of failure⁹. Thus the continued search for possible substitutes would seem justified.

Mineral substances including deproteinized bone¹⁰, apatite,¹¹ and Plaster of Paris^{12,13}, have been used to fill bone defects. Hurley and Losee¹⁴ claimed that ethylene diamine extracted bone could be used clinically on the basis of host acceptance. Hurley, Zeier and Stinchfield¹⁵ reported on the use of this material in 149 humans stating that it served as a space filler and acted as a trellis for organizing granulation tissue and new bone. Stewart¹⁶ and Peltier¹⁷ used Plaster of Paris to fill defects in dog radii and found little foreign body reaction and no stimulation or inhibition of bone formation. Absorption of the material was apparently unrelated to new bone formation.

Recently there have been favorable reports on decalcified bone implants,

hence the emphasis on this material in the present study. Ray¹¹ and Ghormely¹⁸ reported that decalcified bone compared favorably with a variety of grafting materials¹⁹ and more recently Murray²¹ removed the periosteum of rat calvaria and implanted decalcified bone and deproteinized bone adjacent to the parietal bone. All of 38 decalcified bone implants fused, but only 10 of 74 deproteinized bone implants fused to the parietal bone in the time studied. Furthermore, there was less cellular reaction and more new bone about the versenate treated material. Nishi²² also recommended the use of heterogenous bone for transplantation after decalcification. He felt that the organic substances acting as antigens disappear with the calcium components, and has used this material several times successfully in humans. In an early report, Senn²⁰ stated that the use of antiseptic decalcified bone was the best substitute for living bone and the restora-

From the Department of Orthopedics.

Supported in part by N.I.H. Grant No. 47242.

tion of bone substance. His material was demineralized with dilute HCl. Ghormely¹⁸ also stated early that the process of reducing the calcium content of bone would hasten its union in a transplanted position due to a greater ease of permeability and a chemical change in the material.

Little has been written concerning the use of purified collagen implants in bone defects. In this study, collagen implants were compared with decalcified bone, noting the fact that there are differences between bovine skin collagen and decalcified rat bone, such as the content of mucopolysaccharides and molecular configuration. Mineralization of collagen has been reported *in vitro*;^{23,24} however, Sobel²⁵ states nucleation of calcium crystals is activated by mucopolysaccharides and needs a source of energy. Gutman²⁶ implies that mineralization requires a complex of calcium, phosphorus, mucopolysaccharides and collagen as well as cell differentiation. It has been said that the probability of calcification occurring strictly according to physico-chemical mass law phenomena is nil²⁷.

MATERIALS AND METHODS

Defects in the rat radii were made as follows: After suitable preparation, a 1.0cm. incision was made through skin and fascia on the posterolateral aspect of the midforearm. With a mosquito forceps the interval between the brachioradialis and extensors of the forearm was then developed to the radius. Using a six pronged human lacrimal duct, self-retaining retractor to maintain exposure, a 3mm. osteoperiosteal section of the radius was removed with a slow speed dental drill and the bed was irrigated with isotonic saline. The implant was placed between the bone ends, and the skin was closed with a single horizontal mattress suture of 0000 silk.

The decalcified bone was prepared by immersion of whole adult rat radii in versene at pH 4.3 followed by an isotonic saline wash, and storage at 40°F. The Plaster of Paris cylinders were made by forcing fine dental plaster through a #18

needle and cutting the plugs to size after sterilization. The fresh autogenous bone was taken as a plug from one radius, stripped, fragmented, and placed in a defect in the opposite radius. The collagen was bovine skin collagen prepared by the Armour Company.

In the first series of rats subjected to surgery, the effects of age on the healing of the defects was studied. The operation was carried out on rats 33, 48, 60 and 90 days of age. Three months after surgery, X-rays and histological sections showed that defects had failed to heal and typical pseudarthroses were present in every instance.

In the next series, 204 operations were done in 102 male Long-Evans rats between 58-60 days of age and 180 ± 20 grams body weight. Of these, 154 specimens were available for the final study and were divided into five groups according to the types of implants used (Table I). Group I was a control group of 31 defects without implants; group II with 31 defects was implanted with collagen; group III with 32 defects was implanted with Plaster of Paris; in group IV with 31 defects, fresh autogenous bone was used, and group V with 29 defects was implanted with decalcified bone.

One day post-operatively and at two week intervals thereafter, roentgenograms were taken of each animal. At two week intervals, representative animals from each group were sacrificed and the specimen consisting of radius and ulna was removed. These were fixed in 10% formalin, imbedded in celloidin, sectioned, and stained with hematoxylin and eosin. (Fig. 1 through 6 and 7 through 10).

TABLE I

Group	Defects	Implant
I	31	CONTROL, NO IMPLANT
II	31	COLLAGEN
III	32	PLASTER OF PARIS
IV	31	AUTOGENOUS BONE
V	29	DECALCIFIED BONE

It can be seen in the X-rays of these groups that ossification in the defects of controls and those implanted with collagen or plaster is absent or minimal, whereas defects implanted with autogenous or decalcified bone exhibit considerable amounts of new bone. It can also be seen in the longitudinal sections of the rat radii that typical fibrous

pseudarthroses are present between the cut ends in figures 7 and 8, whereas copious amounts of viable bone are present in the specimens shown in figures 9 and 10. These are a control, and defects implanted with plaster, autogenous bone, and decalcified bone respectively.

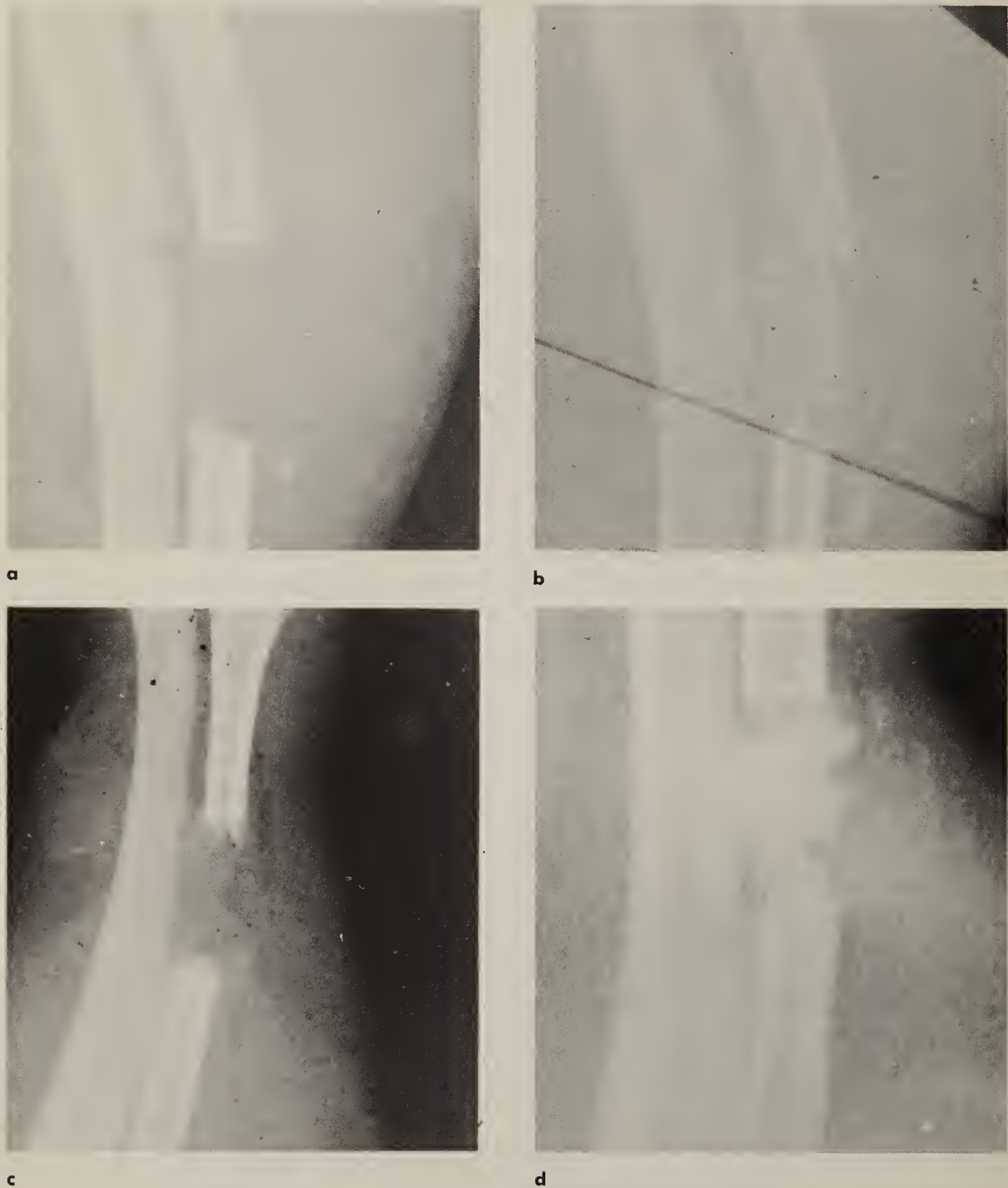
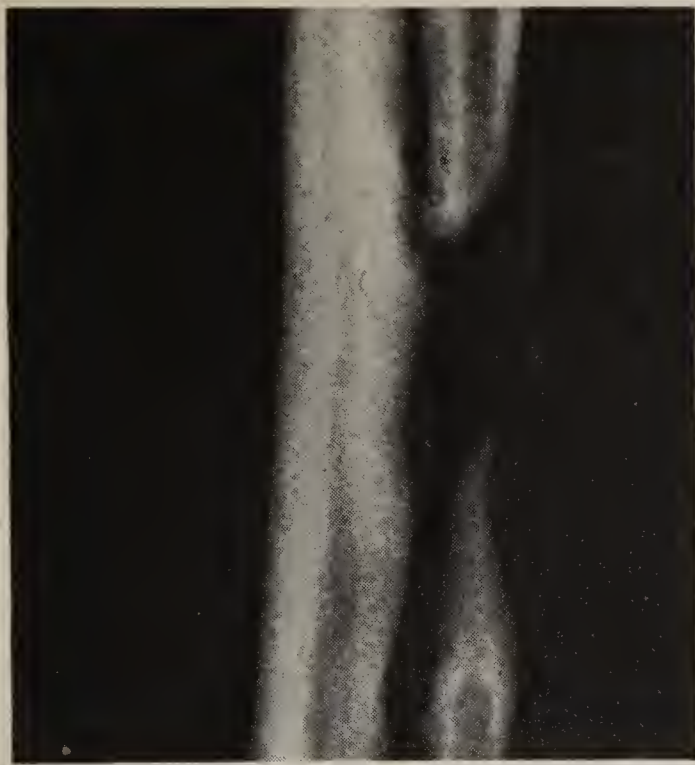
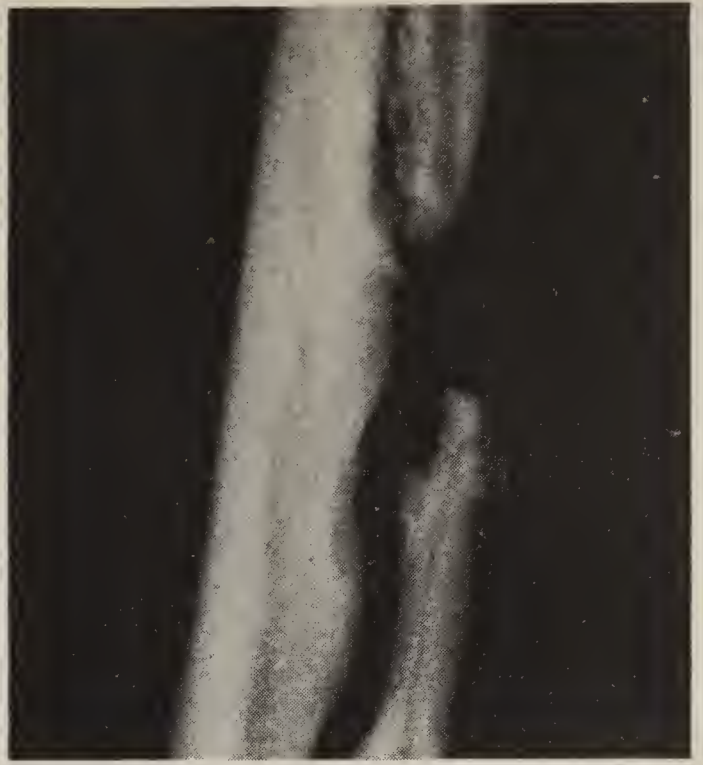


Fig. 1.—Figure 1 shows X-rays of the defects one day after the implants were inserted. 1a contains collagen, 1b is a plaster implant, 1c demineralized bone, and 1d autogenous bone.

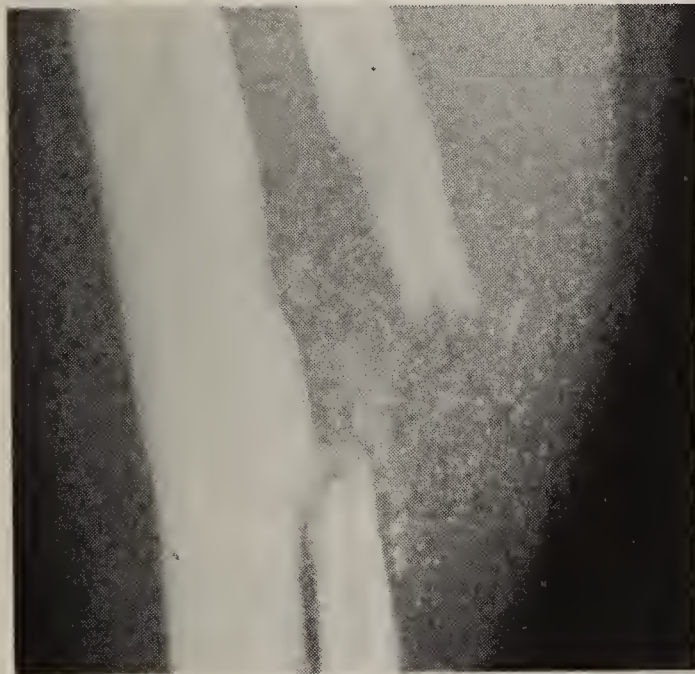


a



b

Fig. 2.—a and b, are six week specimens of control defect without implants.

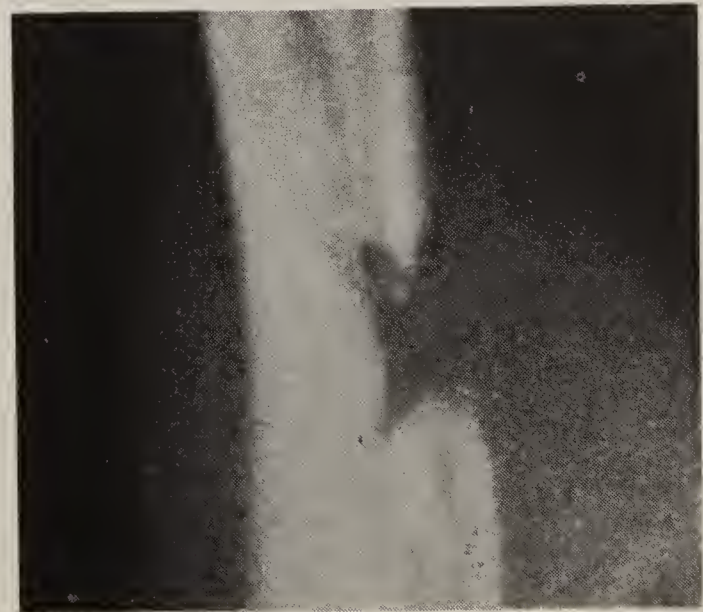


a

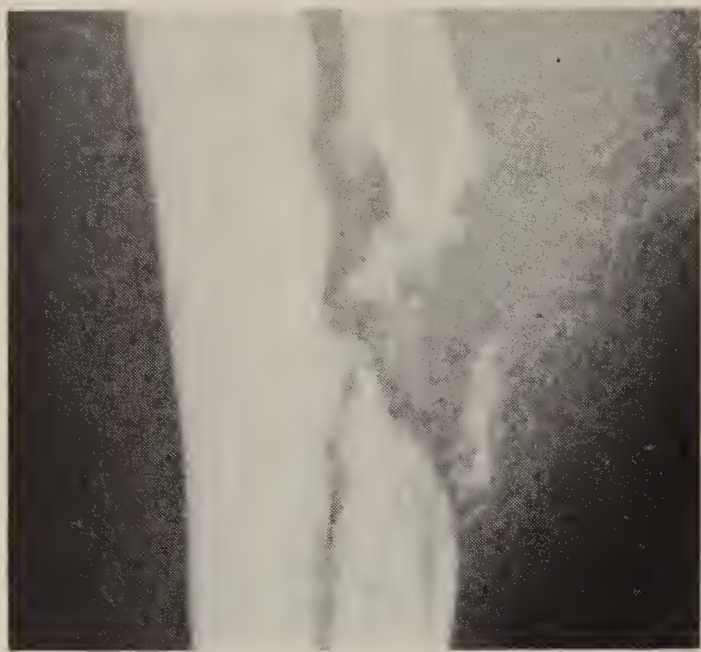


b

Fig. 3.—a, b, and c are six to eight week specimens of defects implanted with plaster. a, corresponds to the specimen in 1 b.



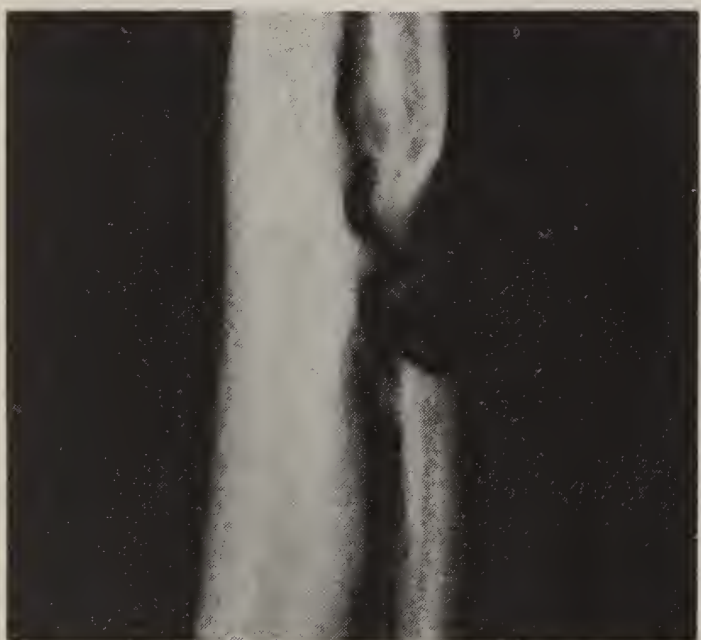
c



a



b

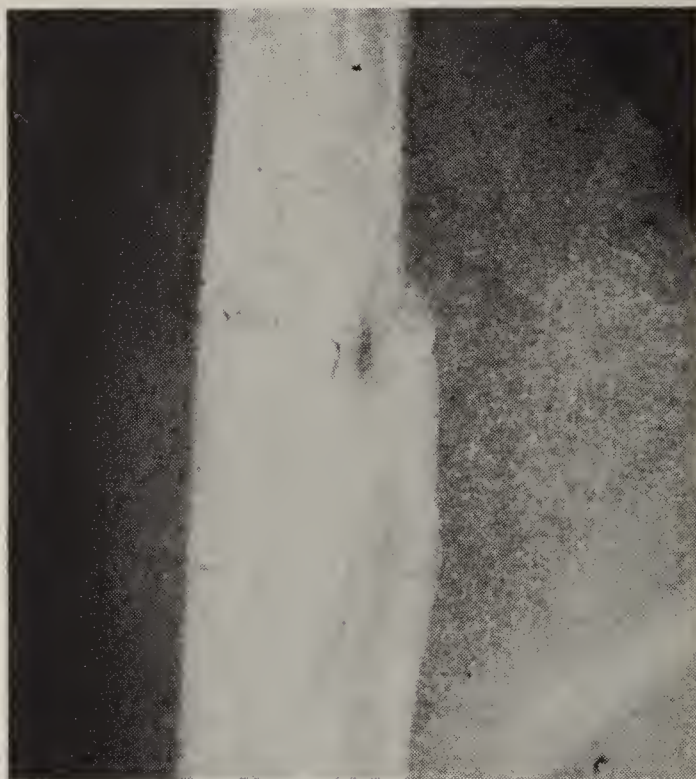


c

Fig. 4.—a, b, and c, are six week specimens of defects implanted with collagen. a, corresponds to the specimen in 1a.



a

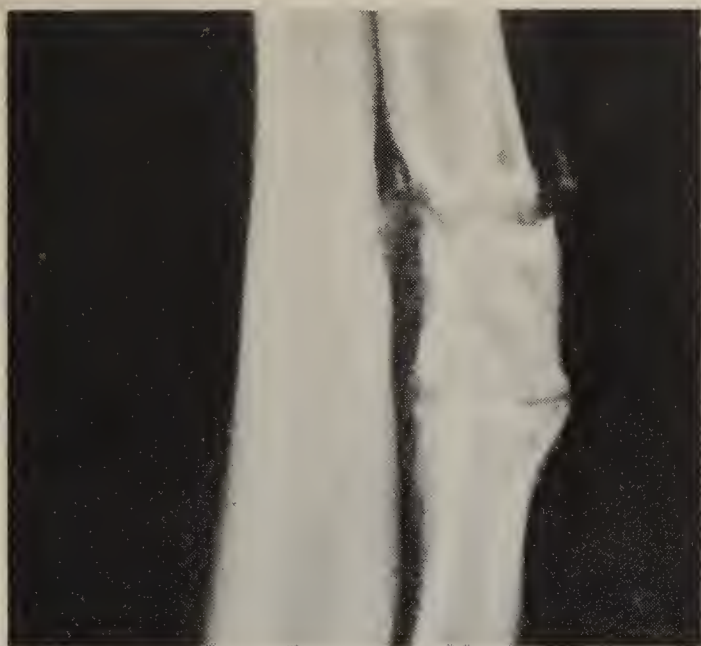


b



c

Fig. 5.—a, b, and c, are six to eight week specimens of defects implanted with autogenous bone. a, corresponds to the specimen in 1d.



a



b



c

Fig. 6.—a, b, and c, are six to eight week specimens of defects implanted with demineralized bone. a, corresponds to the specimen in 1c.



Fig. 7.—The histologic section of the six week control defect shown in 2a. Note the pseudoarthrosis with collagenous fibers perpendicular to the long axis of the radius.

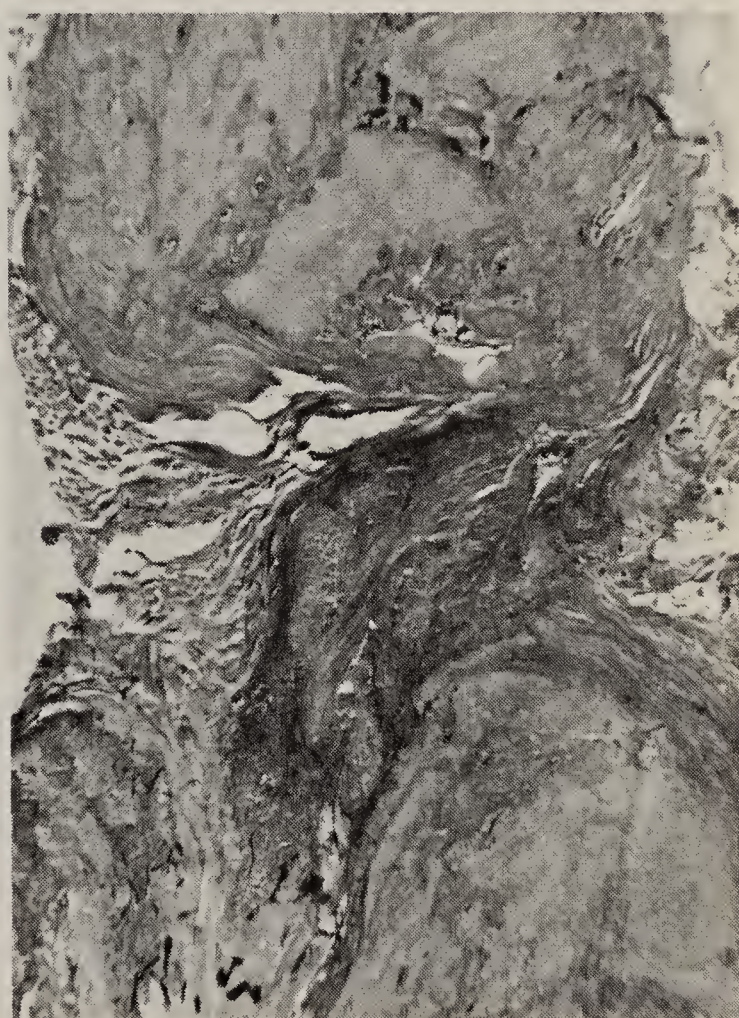


Fig. 8.—The histologic section of the six week plaster implantation shown in 3a.



Fig. 9.—The histologic section of the six week specimen implanted with autogenous bone, same as 5a. Note the abundant new bone formation.

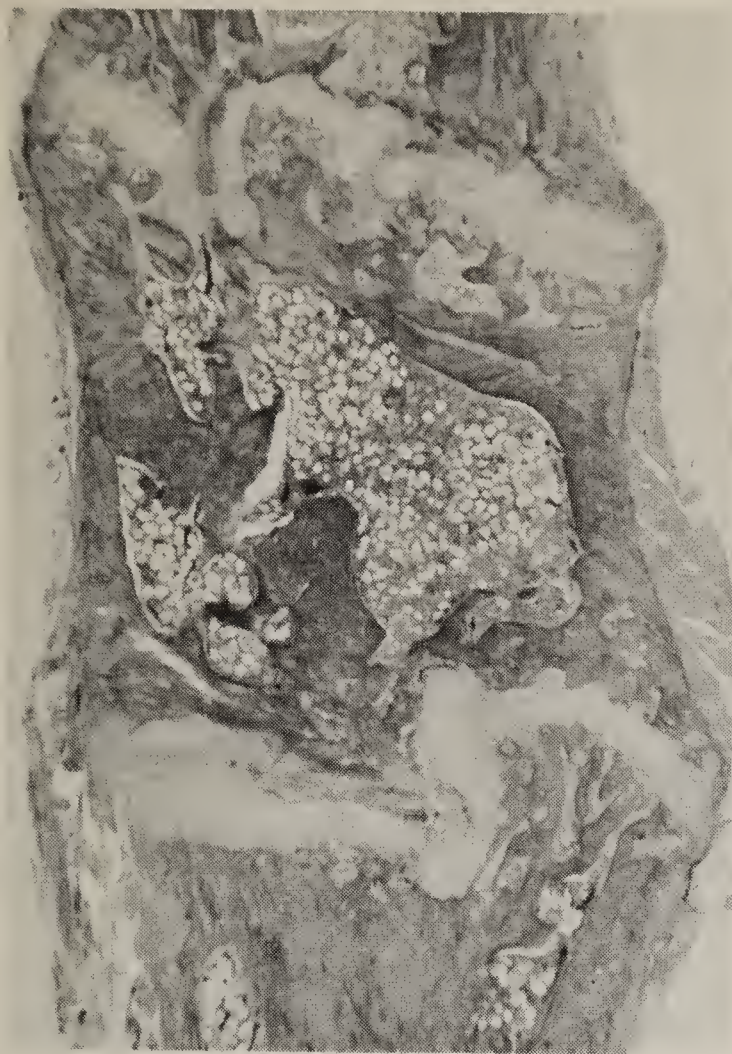


Fig. 10.—The histologic section of the six week specimen implanted with demineralized bone. Same as 6a. Note the new bone with viable marrow between the cut ends of the radius.

RESULTS

The results were evaluated on the basis of the roentgenograms and histological sections. The X-rays were judged according to the presence of union, radio-ulnar fusion, ossification of the graft or defect, resorption of the implanted material, and sclerosis and blunting of the bone ends. Roentgenographically, union occurred in 3 instances with decalcified bone, and in one instance with autogenous bone. Radio-ulnar union occurred 5 times with autogenous bone, 4 times with decalcified bone and 4 times with plaster. Active calcification and ossification were most marked with decalcified and autogenous bone. Sclerosis, blunting, and atrophy of the bone ends

was least marked when decalcified bone was used. Resorption of the Plaster of Paris began early and appeared to be complete by 8 weeks in all cases. Resorption of autogenous bone was slower and more difficult to evaluate due to simultaneous new bone formation.

The histologic sections were evaluated by grading the 6 and 8 week defects 1+, 2+ or 3+ on the basis of new bone formation by observers not familiar with the study. Table II indicates the number of cases in each group with 1+, 2+ or 3+. It is seen that new bone formation is minimal in the control and collagen groups and most marked when autogenous or decalcified bone was used. An attempt was also made to evaluate resorption of the implant histologically. This was difficult in groups IV and V

TABLE II
NEW BONE FORMATION
AT 6-8 WEEKS

Group	+	++	+++
I CONTROL	15/16	1/16	—
II COLLAGEN	11/15	3/15	1/15
III PLASTER	7/15	6/15	2/15
IV AUTOGENOUS BONE	—	2/15	13/15
V DECALCIFIED BONE	1/16	3/16	12/16

TABLE III
EXCESSIVE CONNECTIVE TISSUE
AT 6-8 WEEKS

Group	Percent	Number of Cases
I CONTROL	88%	14/16
II COLLAGEN	80%	12/15
III PLASTER	66%	10/15
IV AUTOGENOUS BONE	12%	2/15
V DECALCIFIED	25%	4/16

due to concurrent revascularization and new bone formation. The plaster, however, was completely resorbed in all instances by 8 weeks, and the collagen was poorly resorbed.

Table III lists the number and percentage of cases in each group which had excessive connecting tissue formation. Autogenous bone, and decalcified bone were also best in this regard.

SUMMARY

In conclusion, it may be stated that demineralized bone was superior to collagen, Plaster of Paris, and the control group, in effecting bony healing,

when used in rat radius defects as described. It compared favorably with autogenous bone by the criteria of comparison used.



REFERENCES

1. Gallie, W. E., and Robertson, D. E.: The Repair of Bone. *Brit. J. Surg.* 7:211, 1919-20.
2. Brooks, B., and Hudson, W. A.: Autogenous and Homogenous Transplantation of Bone in Dogs. *Arch. Surg.* 1:284, 1919.
3. Siffert, R. S.: Experimental Bone Transplants. *J. Bone & Joint Surg.* 37A:742, 1957.
4. Abbott, L. C., et al.: A Comparison of Cortical and Cancellous Bone as Grafts. *J. Bone & Joint Surg.* 29:381, 1947.
5. Henderson, M. S.: Massive Bone Grafts in Ununited Fractures. *J. Am. Med. Assn.* 107:1104, 1936.
6. Hutchison, Jon: The Fat of Bone Autographs and Homographs. *Brit. J. Surg.* 39:552, 1952.
7. Heslop, A.: Study of Osteocyte Survival and Host Reaction Using Autogenous and Homogenous Bone and Grafts. *Brit. J. Exper. Path.* 41:260, 1960.
8. Chase, S. W., and Herndon, C. H.: The Fate of Autogenous and Homogenous Bone Grafts (Historical Review). *J. Bone & Joint Surg.* 37A:809, 1960.
9. Bishop, W. A., Stauffer, R. C., and Swenson, A. L.: Bone Grafts—An End Result Study of the Healing Time. *J. Bone & Joint Surg.* 29:961, 1947.
10. Rosomoff, H. L., Hurley, L. A., and Losee, F. L.: Cranial Reconstruction With Ethylen-Diamine treated Bone. *Am. J. Surg.* 97:721, 1959.
11. Ray, R. D., and Ward, A. A.: Report on Studies of Basic Calcium Phosphate in Bone Replacement. *Surg. Forum* 2:429, 1951.
12. Key, J. A.: The Effect of a Local Calcium Depot on Osteogenesis and the Healing of Fractures. *J. Bone & Joint Surg.* 16:176, 1934.
13. Edberg: Some Experiences of Filling Osseous Defects with Plaster. *Acta Chir. Scand.* 16:176, 1934.
14. Hurley, L. A., and Losee, D. C.: Anorganic Bone—The Chemistry, Anatomy, and Biologic Reactions. *Mil. Med.* 121:101, 1957.
15. Hurley, L. A., Zeier, F. G., and Stinchfield, F. E.: Anorganic Bone Grafting. *Am. J. Surg.* 100:12, 1960.
16. Stewart, W. J.: Experimental Bone Regeneration. *Surg., Gyne., and Ob.* 59:967, 1934.
17. Peltier, L.: *Surg. Forum* 6:556, 1955; 8:571, 1958. Peltier, L.: *Clin. Orth.* V21:1, 1961. Peltier, L.: *Ann. Surg.* 146:61, 1957.
18. Ghormely, R. K., and Stuck, W. G.: Experimental Bone Transplantation. *Arch. Surg.* 28:742, 1934.
19. Freiberg, R.: Unpublished Data.
20. Senn, N.: On the Healing of Aseptic Bone Cavities by Implantation of Anti-Septic Decalcified Bone. *Am. J. Med. Sc.* 98:219, 1889.
21. Murray, J. W. G., Lavine, L. S., and Warrent, R. F.: A Comparative Study of the Healing of Organic and Anorganic Bone Grafts. *N. Y. State J. Med.* 60(2):3617, 1960.
22. Nishi, Nakuna, and Shoji: Transplants of Heterogenous Bone. *J. Bone & Joint Surg.* 42A:543, 1960.
23. Santamon M. S.: The Calcification of Collagen. *J. Molec. Biol.* 1:55, 1959.
24. Glimcher: Current Theories of Bone Salt Formation. *Proc. Nat. Acad. Sc.* 43:860, 1957.
25. Sobel, Lawrence, and Burger: Nuclei Formation and Crystal Growth in Mineralizing Tissue. *Trans. N. Y. Acad. Sc.* 22(4):233, 1960.
26. Gutman, A. B.: Current Theories of Bone Salt Formation. *Bull. Hosp. Joint Surg.* 12:74, 1951.
27. Neuman, W. F., and Neuman, M. W. *Chem. Rev.* 53:1 1953.

REVIEW ARTICLE

UREA AND UREASE IMMUNITY

ANDREW THOMSON, M.D.

Urea, whether endogenous or parenterally administered, is a freely diffusible molecule which rapidly achieves equilibrium in body water. Isotopic urea studies have recently shown that equilibrium is achieved within three hours and that the urea pool represents 56-70% of body weight.¹ Urea has long been considered metabolically inert and this assumption led to the studies of isotopic urea for the measurement of total body water.² However, one of the requirements for the measurement of total body water is that urea is not metabolized and recent evidence shows that 20-25% of endogenous urea is hydrolyzed in the gastrointestinal tract. In normal man hydrolysis of endogenous urea occurs at a relatively constant rate of .3 gm. urea/hr. This amounts to 7.2 gm. urea per day and is the equivalent in nitrogen content to 11 gm. per day of NH_4Cl .¹ This quantity of ammonia is certainly sufficient to induce coma or precoma in many patients with relatively far advanced liver disease. Urease is the only enzyme known to accomplish this hydrolysis *in vivo* and it is believed to be entirely of plant or microbial origin.³ Urease in man is thought to be entirely of microbial origin and localized principally in the distal ileum and proximal colon. This urease activity can be eliminated by antibiosis and, following antibiotic therapy, labeled urea is completely recovered in the urine.¹

Ammonia is detrimental to patients with liver disease and elevated blood ammonia concentrations are frequently associated with hepatic coma⁴. Clinicians

are fully aware that the correlation is often very poor, yet additional evidence continues to incriminate ammonia as one of the most important factors. The rapid breakdown of glutamine to ammonia in stored blood and its propensity to produce coma is a well known clinical fact. The recent demonstration of the

From the Department of Medicine.
Supported in part by Public Health Grant
No. AM07894-GM.

reciprocal relationship of serum ammonia and serum potassium by Baertl and associates explains very graphically why thiazide diuresis frequently precipitates coma⁵. Perhaps the best evidence is provided by the universal therapy of protein restriction and antibiotics. Other ingenious surgical procedures such as McDermott's recent report of 3 ileo-proctostomies⁶ or reports of total colectomies are attempts to exteriorize or eliminate urease sources capable of urea hydrolysis.

Under normal circumstances, ammonia in the gastrointestinal tract resulting from the action of bacterial enzymes upon proteins and their derivatives, is carried by way of the portal circulation to the liver where it is enzymatically converted to detoxification products, primarily urea^{4,7,8}. The blood ammonia level represents a balance between ammonia production in the gastrointestinal tract, removal by the liver, uptake and

release by the tissues, and production and excretion by the kidneys⁴. (See Fig. 1). Experiments using C-14 urea in laboratory animals and birds have shown that substantial reduction in urea hydrolysis can be achieved by antibacterial levels as low as 100 ppm. Simultaneously these agents produced a reduction in *in vitro* urease activity and ammonia concentration in homogenates of the combined gastrointestinal tract plus content⁹. This effect of depressing gastrointestinal ureolytic activity and ammonia concentration was also observed in laboratory animals fed barbituric acid, a urease inhibitor^{10,11}, and in those immunized with crystalline jackbean urease¹². Laboratory animals immunized to crystalline jackbean urease had antibodies in their serum, feces and urine which were capable of hemagglutinating human erythrocytes coated with jackbean urease¹³. These antibodies accounted for a 60% reduction of ureolytic

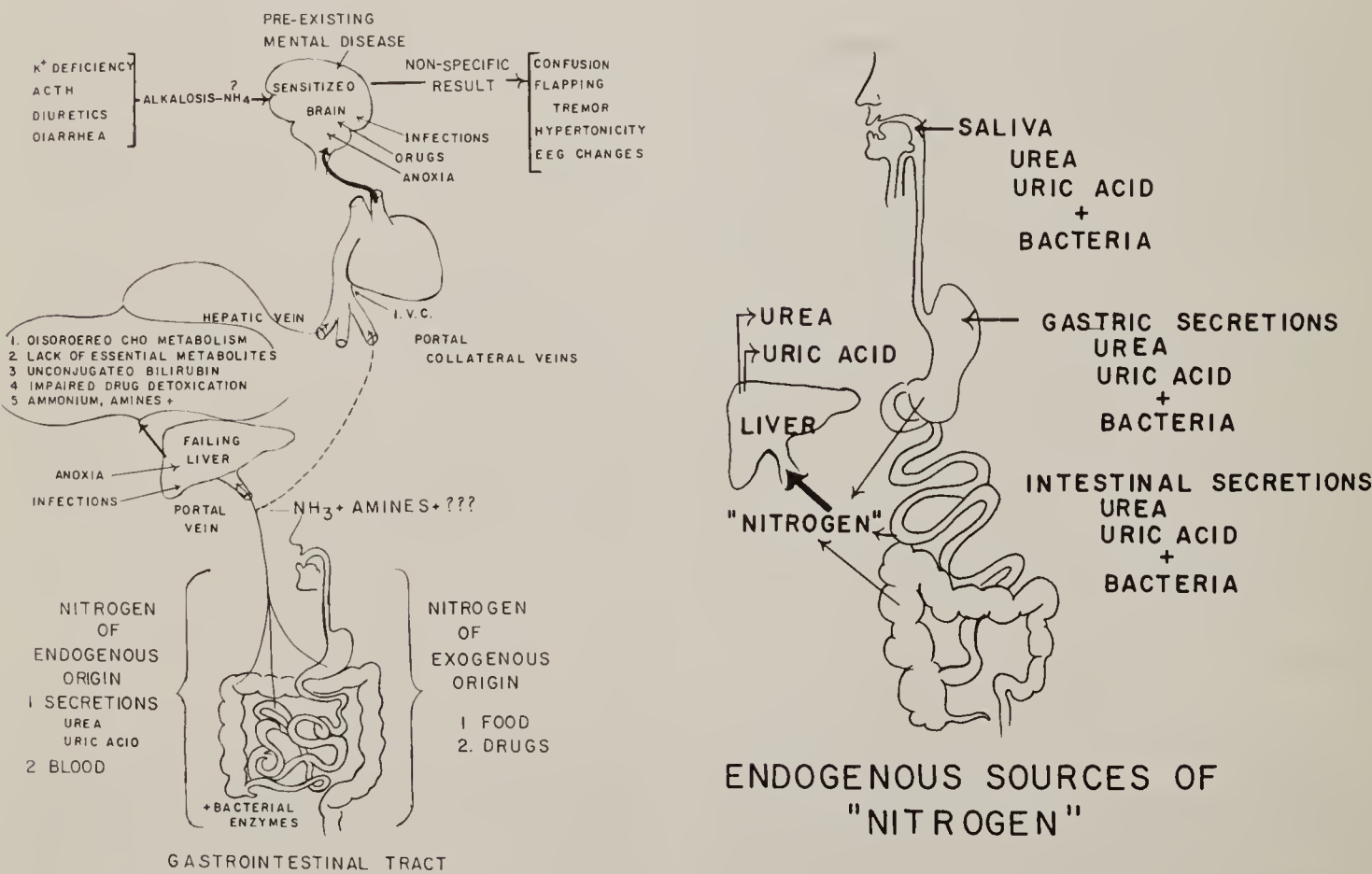


Fig. 1.—Diagrams illustrate some of the most important hepatic agents responsible for hepatic encephalopathy with simplified metabolic pathways.

activity of the gastrointestinal content of immunized animals as compared to controls¹⁴. Similarly the addition of jackbean urease immune serum to normal gastrointestinal content reduced ureolytic activity to a similar extent. This binding of antiurease antibody to urease *in vitro* has been demonstrated by Marucci and Mayer¹⁵.

The above evidence suggests that the urea hydrolytic system can be profoundly altered by jackbean urease immunity. This concept requires antibody to cross into the gastrointestinal lumen where it can specifically bind bacterial urease. Other evidence for the presence of antibody in the gastrointestinal lumen would support this thesis^{16, 17, 18}. The question of how avidly the antibody-urease bond may be is important and has not yet been answered. Antiurease antibody has been shown to bind for at least one hour and thereby neutralize urease activity *in vitro* without significant elution of antibody.

Prior to the studies using jackbean urease as an antigen in man, toxicity studies were conducted in approximately 500 animals of 9 different species. Provided the dose of urease was kept below the midlethal level (LD50), there was no evidence of toxicity or pathological effect either in acute or chronic exposure¹⁴.

Urease was crystalized from jackbean meal using Sumner's procedure with Dounce's modifications¹⁹. Three to five crystallizations were carried out on all enzyme to be used for human experimentation. This resulted in a relatively lower yield of enzyme but produced a highly purified, highly antigenic enzyme. Urease has a molecular weight of 483,000, and is a water soluble globulin. (See Fig. 2). The optimal antigenic level in man ranges between 60-100 ug. given subcutaneously.

All of the patients immunized with urease had far advanced liver disease and six had surgically produced portocaval shunts. Four Sumner units of urease (40 ug. protein) subcutaneously injected as a primary immunizing dose

1. PROPERTIES

- a. Globulin - colorless octahedra
- b. Mol. wt. 483,000
- c. Turnover No. 460,000
- d. Isoelectric pt. pH 5.0-5.1
- e. SH groups - no Molisch rxn

2. OCCURRENCE

Bacteria, molds, higher plants

3. ACTION AND SPECIFICITY

- a. Urea $\longrightarrow$ CO₂ and NH₃
NH₄ carbamate if unbuffered.
- b. Absolutely specific

4. KINETICS

Zero order reaction

5. INACTIVATION AND INHIBITION

- a. Highly unstable when pure
- b. Contains hemagglutinin if not pure

Fig. 2.—Crystalline Urease.

produced serum levels of antiurease which were detectable by hemagglutination and precipitin reactions. There was no toxic or anaphylactic response. On two occasions, patients gave a secondary response to the primary challenge, suggesting that they had been previously sensitized to a comparable antigen. Secondary immunizing doses of 8-10 units were well tolerated by all of the patients without evidence of ammonia intoxication. Detectable antibody persisted for at least 6 weeks. Although there was variation between individuals, all of the patients responded with antibody production to the secondary challenges when given at 3-4 week intervals. Detectable antibody titers were measurable for at least 6 weeks. Six of the eight patients were able to increase their daily protein intake from 80-300% without any evidence of an increase in their fasting venous ammonia levels. These six patients showed marked clinical improvement without antibiotic therapy. Five patients demonstrated marked rises in the serum albumen levels, and subsequent diuresis. More detailed reports are in press^{20,21,22}. A secondary immunologic challenge of 20 units of urease in two patients was followed by signs of ammonia intoxication 3-4 days after injection, when antigen disappearance and antibody depression are expected to oc-

RESPONSES TO FIRST, SECOND AND THIRD INJECTIONS OF I BGG RABBIT # 977

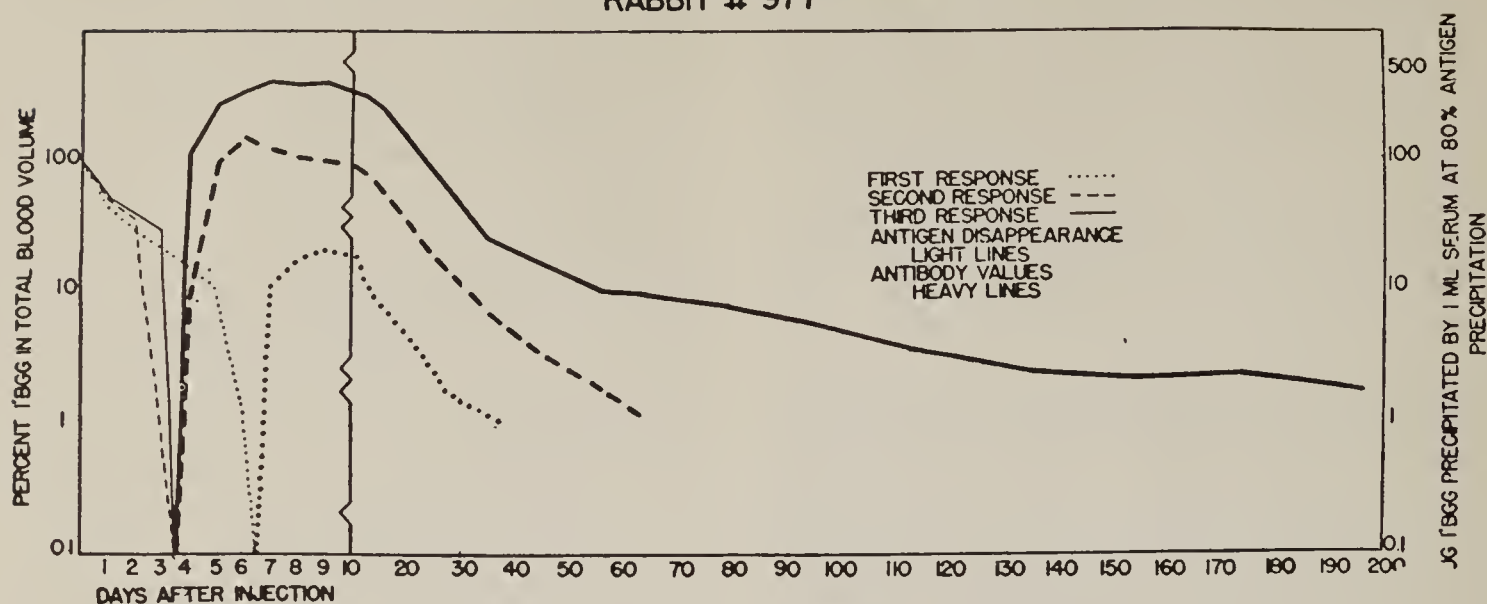


Fig. 3.—This chart shows radioactive labeled antigens disappearance in light lines on the primary, secondary, and tertiary challenge. The heavier lines represent antibody appearance following the antigen disappearance.

cur. As demonstrated by Dixon, et al., radio-labeled soluble protein will reach equilibrium and then disappear on the fourth day very suddenly, followed by the appearance of antibody²³. (See Fig. 3).

Urease immunity is a new approach to the problem of controlling the action of gastrointestinal flora by a physiological mechanism. This immunity has been sustained as long as two years in man without any detrimental effect, except the development of temporary coma with overdosage. Urease immunity has the

advantage of continuously depressing urea hydrolysis. It does not depend upon ingestion of food or drugs and thus is operative during periods of fasting or between meals when the liver may contain limited supplies of substrates required for ammonia detoxification²⁴. Urease immunity should be studied for more extended periods in larger series of patients to see if prolonged control of ammonia production will improve longevity in these nutritionally-crippled patients.



REFERENCES

1. Walser, M., and Bodenlos, L. J.: Urea Metabolism in Man. *J. Clin. Invest.* 38:1617, 1959.
2. Steffenson, K. A.: Some Determinations of the Total Body Water in Man By Means of Intravenous Injections of Urea. *Acta. Physiol. Scand.* 13:282, 1947.
3. Kornberg, H. L., and Davies, R. E.: Gastric Urease. *Physiol. Rev.* 35:169, 1955.
4. Sherlock, S.: Hepatic Coma. *Ann. Rev. Med.* 11:47, 1960.

5. Baertle, J. M., Sancetta, S. M., and Gabuzda, G. J.: Relations of Acute Potassium Depletion to Renal Ammonium Metabolism in Patients with Cirrhosis. *J. Clin. Invest.* 42:696, 1963.
6. McDermott, W. V., Jr., Victor, M., and Point, W. W.: Exclusion of the Colon in the Treatment of Hepatic Encephalopathy. *New Eng. J. of Med.* 267:850, October, 1962.
7. Krebs, H. A.: Metabolism of Amino Acids: Deamination of Amino Acids. *Biochem. J.* 29:1620, 1935.
8. McDermott, W. V., Jr.: Metabolism and Toxicity of Ammonia. *New Eng. J. Med.* 257:1076, 1957.
9. Visek, W. J., Baron, J. M., and Switz, D. M.: Urea Metabolism and Intestinal Ureolytic Activity of Rats Fed Antimicrobial Agents. *J. Pharm. Exp. Therap.* 126:359, 1959.
10. Visek, W. J., Jacobson, A. I., Iwert, M. E., and Alvares, A. P.: Depression of Urease Activity in the Gastrointestinal Tract by Chemical Agents. *Fed. Proc.* 20:370, 1961.
11. Harbers, L. H., Alvares, A. P., Jacobson, A. I., and Visek, W. J.: Effect of Barbituric Acid and Chlortetracycline Upon Growth, Ammonia Concentration and Urease Activity in the Gastrointestinal Tract of Chicks. *J. Nutr.* 80:1, 1963.
12. Dang, H. C., and Visek, W. J.: The Effect of Urease Injections On Body Weights of Growing Rats and Chicks. *Proc. Soc. Exp. Biol. Med.* 105:164, 1960.
13. Visek, W. J., Iwert, M. E., and Burrows, W.: Detection of Antibody to Urease by Hemagglutination. *Proc. Soc. Exp. Biol. Med.* 109:54, 1962.
14. Dang, H. C., and Visek, W. J.: Some Effects of Urease Administration on Laboratory Animals. *Am. J. Physiol.* (In press).
15. Marucci, A. S., and Mayer, M.: Quantitative Studies on the Inhibition of Crystalline Urease by Rabbit Antibody. *Arch. Biochem. and Biophys.* 54:330, 1955.
16. Gabuzda, G. J., Jackson, G. G., and Gregsby, M. W.: Effect of Aureomycin Upon Nitrogen Balance and Urinary Riboflavin Excretion in Undernourished Man. *J. Clin. Invest.* 31:631, 1952.
17. Horowitz, M. I., and Hollander, F.: Occurrence of Plasma Proteins in Gastric Mucus and Other Secretions. *Ann. N. Y. Acad. Sci.* 99:Art. 1, 67, 1962.
18. Burrows, W., and Havens, I.: Studies on the Immunity to Asiatic Cholera. V. The Absorption of Immune Globulin From the Bowel and its Excretion in the Urine and Feces of Experimental Animals and Human Volunteers. *J. Inf. Dis.* 82:231, 1948.
19. Dounce, A. L.: An Improved Method For Recrystallizing Urease. *J. Biol. Chem.* 140:307, 1941.
20. Thomson, A., Iwert, M. E., and Visek, W. J.: Immunity to Jackbean Urease in Patients with Hepatic Insufficiency and Ulcerative Colitis. *Fed. Proc.* 21:265, 1962.
21. Visek, W. J., and Thomson, A.: Some Effects of Urease Immunity in Hepatic Insufficiency. *J. A. M. A.* (In press).
22. Thomson, A., and Visek, W. J.: Some Effects of Induction of Urease Immunity in Hepatic Insufficiency. *Amer. J. Med.* (In press).
23. Dixon, F. J., Maurer, P. H., and Deichmiller, M. P.: Primary and Secondary Anamnestic Antibody Response of Rabbits to Heterologous Serum Protein Antigens. *J. Immun.* 72:179, 1954.
24. Krebs, H. A., and Henseleit, K.: Untersuchungen über die Harnstoffbildung im Tierkörper. *Zeitschr. Physiol. Chem.* 210:33, 1932.

ABSTRACTS AND REVIEWS

OF PUBLICATIONS BY THE STAFF

The Role of Fluctuations of Sodium Balance in The Regulation of Body Sodium Stores. D. BALDWIN, H. D. LANDAHL and T. B. SCHWARTZ: *Metabolism*, Vol. 12, No. 11 November, 1963.

Sodium excretion in the presence of a constant daily intake takes the form of 2 to 5 day cyclical fluctuations, this pattern becoming increasingly apparent at higher sodium loads. A rough correlation exists between these cyclical fluctuations of sodium excretion and water excretion at normal or high sodium intakes. The amplitude of these oscillations of sodium excretion is a linear function of the magnitude of the daily sodium chloride load (or mean excretion) in the short run in all normal subjects studied. These cyclical fluctuations can be demonstrated to be non-random and predictable from one normal subject to another when the daily intake is increased from a lower to a higher level, or decreased from a higher to a lower level. The exaggerated fluctuations of sodium excretion that accompany a low sodium intake following a period of chronic sodium loading rapidly dampen down to the minimal fluctuations predicted from nonpreloaded low intake studies. Comparable cyclical fluctuations can be demonstrated in adrenal insufficient subjects maintained with adrenal steroid replacement therapy. These observations strongly suggest that these cyclical fluctuations represent a servo-system derived from alternating lags in sodium retention and sodium excretion, and significantly concerned with the regulation of total body sodium. Such a regulatory system could not be derived from fluctuating adrenal steroid secretion.

Left Ventricular End-Diastolic State in Man, R. A. CARLETON, BY INVITATION and J. S. GRAETTINGER, *Journal of Laboratory and Clinical Medicine* 62:865, 1963.

In physiologic studies in healthy man the readily measurable left ventricular end-diastolic pressure (P_{ED}) has been used to characterize the end-diastolic state as it relates to systolic performance. Data obtained in animals and from muscle strips, however, would suggest that systolic performance is related to initial fiber length and, in the heart, to ventricular end-diastolic volume (V_{ED}). The relationship between P_{ED} and V_{ED} in active man is the subject of this study.

Measurements of P_{ED} , V_{ED} (sodium ascorbate dilution), and output were made in 9 asymptomatic patients, of whom 5 had evidence of minimal heart disease, in 2 patients with complete heart block paced at varying rates, and in 11 with severe mitral stenosis. All were studied at rest and exercise and during epinephrine infusion in 7.

The relationship between P_{ED} and V_{ED} varied widely among the patients. Moreover, except in the patients with heart block, no single P-V curve existed for any patient. The relationship of P_{ED} to stroke work was not consistent among different patients nor in the response of the same patient to epinephrine or exercise. We conclude that P_{ED} is an inadequate measure of end-diastolic state.

Conversely, in all patients, stroke work responded concordantly to changes in V_{ED} , expressed as end-diastolic circumference (C_{ED}) of a spherical left ventricle, both in a given patient and among the various patients. This heterometric ventricular work response occurred at rest, at exercise, and with epinephrine. In patients with severe mitral stenosis, however, stroke work changed significantly less for a given change in C_{ED} than in the other patients.

P_{ED} is merely a resultant of V_{ED} and the impedance to ventricular filling. C_{ED} , as an estimate of diastolic fiber length in man, provides an important parameter of ventricular function studies.

Carcinoma of the Bile Ducts. E. L. STROHL, W. H. REED, W. G. DIFFENBAUGH, and R. E. ANDERSON: *Archives of Surgery*, Vol. 87, pp. 567-577, October 1963.

Carcinoma of the major extrahepatic and intrahepatic bile ducts, although infrequently encountered, merits consideration in every patient having obstructive jaundice. The correct pre-operative diagnosis is seldom made. The operative diagnosis is difficult because of the limited anatomical boundaries and the difficulty of thorough exploration of the ductal system. Malignant tumors of the ducts produce complete obstruction early in the course of the disease, in most instances. The proximity of the vital structures permits the tumors to grow to an inoperable state in a short period of time following the onset of symptoms. This study concerns malignant tumors of the extrahepatic and intrahepatic ducts, exclusive of the ampulla of Vater.

A characteristic feature of carcinoma of the bile ducts is the rapid onset of symptoms. In our series, the interval between the onset of symptoms and the hospital admission ranged between one and eight weeks. Jaundice was present in 20 of our patients, or 76%. The icteric index was elevated between 80 and 300 units, with an average of 169 units.

Pain was a prominent symptom in 13 of our patients, or 50%. Weight loss was a prominent symptom. A palpable liver is usually present and is rarely tender.

Carcinoma of the bile ducts merits consideration in every person having obstructive jaundice, particularly in the elderly patient.

The most common type of bile duct carcinoma is a firm, circumscribed, nodular tumor obstructing the duct. No metastatic involvement is present in about 50% of the patients having bile duct carcinoma.

Pruritus and jaundice, which progress rapidly, are the most prominent symptoms.

Pain, intermittent, or of the colic type, is present in one-half of the patients.

Treatment should accomplish resection of the tumor, if at all possible. Failing resection, some method to relieve the obstruction and deliver bile into the gastrointestinal tract is necessary. External drainage procedures are unsatisfactory.

Intravenous Pancreatography—A Negative Result. J. SILVERMAN, and B. JAY HILL: *Radiology* 81, 596, October, 1963.

Intravenous pancreatography was attempted in nine patients. After administration of 40 mls. of Cholografin and visualization of the gall bladder, large doses of intravenous morphine were given to constrict the ampulla of Vater. This was followed by Cholecystokinin, a potent cholagogue, in an attempt to convert the gall bladder into an internal syringe, filling the pancreatic duct in retrograde fashion. The attempt was unsuccessful in each instance. Greater doses of morphine to increase the sphincteric block were deemed inadvisable.

Acute Cholecystitis. E. L. STROHL, W. G. DIFFENBAUGH, R. E. ANDERSON: *Illinois Medical Journal* 124, #1 pp. 29-34, July, 1963.

A stone impacted in the cystic duct is the usual causal factor of acute cholecystitis. Stones were present in 85.6 per cent of our patients. Obstruction of the cystic duct is followed by distention of the gallbladder. The entrapped bile produces a chemical alteration of gallbladder mucosa, a chemical cholecystitis. It has been shown by Womack and Hefner, experimentally, that the acute inflammatory changes vary in severity in direct proportion to the concentration of the gallbladder bile. The distention of the gallbladder may impair the blood supply. Small areas of gangrene and perforation may follow. Bacteria may invade the damaged tissues by way of the lymphatics, or bacteria may be present in the gallbladder bile which has passed through the liver into the gallbladder. One can obtain positive bacterial cultures in but about 35% of patients having acute cholecystitis. Acute noncalculous cholecystitis, however, may account for 5 to 10 per cent of all reported cases of acute cholecystitis. Many etiologic factors may contribute to such a disease entity. These include bacteria which have passed through the liver with the bile bacteria from the bloodstream, or bacteria which have reached the gallbladder by way of the lymph channels.

Some investigators have placed great importance on the reflux of pancreatic juice as an inciting agent in acute cholecystitis. In addition, the vascular changes of arteriolitis, and the terminal changes of arteriosclerotic disease have been considered as etiologic factors in acute noncalculous cholecystitis. Noncalculous cholecystitis can proceed to gangrene and perforation in a manner similar to that seen in calculous cholecystitis. The attack, however, is more insidious, with no acute episode at the onset. As the pain increases, the area becomes more tender and the gallbladder enlarges. The pathologic changes in acute calculous and noncalculous cholecystitis are similar except for the presence of stones. The time required for the changes to appear in the calculous or noncalculous gallbladder varies with the degree of obstruction of the blood supply to the gallbladder.

It is an interesting historical fact that the first case of gangrene of the gallbladder, with perforation, followed by peritonitis and death, was reported by James Duncan of Edinburgh, in 1844. These complications followed a femoral herniorrhaphy in a young woman, and, were found at autopsy.

Acute cholecystitis, pathologically confirmed, occurs between 8 and 10 per cent of patients having calculous cholecystitis. The disease is found with much greater frequency in the aged patient.

Gangrene and perforation of the acute gallbladder is associated with a high morbidity and mortality, particularly in the aged patient.

In our opinion, early surgery is indicated in acute cholecystitis, after adequate preparation has been carried out. Cholecystectomy is the operation of choice, in a good risk patient, in the early course of the disease. Cholecystostomy will release the obstruction and provide drainage, in the aged or poor risk patient, in an advanced stage of acute cholecystitis. Definitive surgery may be done at a later date, if indicated.

Arterial Emergencies. G. DE TAKATS: *Medical Times*, Vol. 91, No. 10, October 1963.

As pointed out in this brief survey, vascular emergencies arise from three basic sources, namely, embolism, thrombosis and hemorrhage. To this, one may add an acute arterial insufficiency due to a sudden fall in blood pressure in the presence of narrow or segmentally obstructed arteries. This is specially important in areas which stand ischemia poorly and only for a short time, as in the brain, the heart and the kidneys. Restoration of blood pressure to above a critical level thus becomes important. Only in the presence of hemorrhage would a rise in blood pressure be deleterious.

Specialists in this field, whether they are abdominal surgeons, neuro-surgeons, urologic surgeons or vascular surgeons, can only practice their technical skills when the all-important diagnosis is made and rapid transportation to a hospital is arranged. The training of the general practitioner in the recognition of early signs and symptoms of arterial emergencies is obviously the key point to successful management. The removal of an expanding abdominal aneurysm by a trained surgical team supported by skillful anesthesia, is no harder than the early decision to move such a patient from a metropolitan or rural home to a hospital which is equipped for such emergencies. During the last few years, as a result of graduate and postgraduate education, facilities are ready and available within reasonable distance.

Johannes Scultetus. E. L. STROHL: *Archives of Surgery*, Vol. 87, pp. 693-697, September 1936.

Although Johannes Scultetus (1595-1645) has been dead for over three hundred years, there are daily reminders of his ability and skill in the many-tailed binder which is employed by surgeons to this day.

Born John Schulte, in Ulm, Germany, he used the Latin form of his name in his writings, which were in Latin, as was the custom of his era.

Known as one of the most venturesome surgeons of that time, his army experience, during part of the Thirty Year Wars, probably accounted for his daring surgical work on patients.

Surgeons of that period realized the necessity of improving their instruments, tools, and operative methods, and Scultetus undertook the task of standardizing surgical techniques.

His great mechanical ingenuity is demonstrated in his own illustrations of bandages, instruments, and operative procedures, all meticulously described. Paradoxically, his strength might have been his weakness, for the complexity, rather than simplicity of his work, may have lessened the recognition it deserved.

His "Armamentarium Chirurgicum," published posthumously at the Hague, includes not only 100 of Scultetus' observations, but surgical techniques used in the reduction of fractures and dislocations, amputation of limbs, breasts, and other surgical procedures.

Pre- and Postoperative Endoscopic Problems Associated with Cardiovascular Surgery.
P. H. HOLINGER: *Acta Oto-Laryngologica Supplementum* 183—1963.

Many pre- and postoperative problems of cardiovascular surgery are of endoscopic interest and importance. A high incidence of congenital laryngeal cysts, webs, paralyses and laryngoceles is associated with congenital heart disease, accentuating respiratory embarrassment and cyanosis. Unilateral left cord paralysis is found in infants with vascular rings, great vessel transposition, occasionally in tetralogy of Fallot and lesions associated with a rapidly enlarging heart. When laryngeal paralysis is recognized preoperatively or develops during surgery, postoperative problems of aspiration and poor deglutition are increased.

A tracheotomy in the postoperative course of cardiovascular surgery may be required to relieve laryngeal obstruction following prolonged anesthesia intubation; permit frequent tracheobronchial aspiration; provide direct access to the airway for positive pressure respiration; or enable introduction of a tracheal or bronchial support following surgical relief of compression caused by a vascular anomaly.

In patients with congenital or acquired heart disease, bronchoscopic inspection differentiates between cardiac decompensation with pulmonary congestion and intrinsic bronchopulmonary disease when symptoms such as cough, dyspnea, hemoptysis, wheezing, obstructive emphysema or atelectasis develop. The diagnosis of tracheal or bronchial obstruction produced by vascular anomalies, right bronchus trauma following some of the surgical procedures used in transposition operations, and differentiation between retained secretions and cardiac compression as the cause of left lung atelectasis are further indications for bronchoscopy in the pre- and postoperative cardiac patient.

Pleurectomy in the Treatment of Pleural Effusion Due to Metastatic Malignancy.
R. J. JENSIK, M.D., J. E. CAGLE, JR., M.D., F. MILLOY, M.D., C. PERLIA, M.D., S. TAYLOR, M.D., S. KOFMAN, M.D., and E. J. BEATTIE, JR., M.D.: *The Journal of Thoracic and Cardiovascular Surgery*, September, 1963.

From 1957 to 1962, fifty patients were subjected to pleurectomy in an attempt to control rapid re-accumulation of pleural fluid as a result of metastatic carcinoma.

The primary carcinoma was in the breast in 18, lung in 13, and in miscellaneous sites in 11 others. It was unknown in the remaining 8.

The immediate surgical mortality was six per cent although six additional patients failed to survive longer than one month.

Average survival for the remaining 41 patients was 10.4 months; although the figure for the breast group was 14 months. Of the five patients who survived more than two years, all had had previous breast carcinoma.

The ideal candidate for the procedure is the patient with metastatic disease from primary breast carcinoma involving only one pleural cavity, requiring two thoracentesis per week and, if present, having other metastatic foci under control by chemotherapeutic, hormonal, or roentgen therapy.

The Fleece Inn Medical Society. E. L. STROHL: *Surgery, Gynecology & Obstetrics*, Vol. 117, 371-374 September, 1963.

Although organized as the Gloucestershire Medical Society in 1788, the meeting place was in the Parlor of the Fleece Inn, Rodborough, England, and the group became known as the Fleece Inn Medical Society.

The membership consisted of outstanding physicians of the day, the moving force of which was Dr. Edward Jenner, who found the group willing to listen to his discourses on Smallpox, a subject which was taboo at another association of which he was a member.

Another member of the society, Caleb Parry, made a contribution to medicine in his book on "Angina Pectoris," the original of which was read at the Fleece Inn Medical Society.

Public health was a matter of concern at the time (1789) and a member of the society, John Heathfield Hickes, delivered an address on the subject before the group.

The excellent esprit de corps is Jenner's reluctance to publish his observations on angina pectoris for fear of depriving John Hunter of the hope of recovery from the disease from which Jenner believed he was suffering.

Many of Jenner's papers presented before the Fleece Inn Medical Society have been lost, one of which was his observations of the disease of the heart occurring during attacks of acute rheumatism, and had the paper been published at the time it would have established his claim to priority.

Jenner and Parry told us, more than 175 years ago, that anginal pain was associated with thickening of the arteries, but it was not until 1912 that his concept became universal.

One wonders how many valuable theories and observations remain obscure and unrecognized in the archives of small medical groups.

Coordinated Surgical-Chemical Therapy of Cancer. F. E. KNOCK: *J. Am. Med. Assoc.*, 186, No. 6, 558-565, 1963.

Attack aimed at protein coat of DNA by surgical use of sulfhydryl inhibitors plus adjuncts, is being investigated alone and with drugs aimed at DNA, like 5-FU. In 7 of 12 patients, chemotherapy with sulfhydryl inhibitor oxophenarsine plus adjuncts regressed large masses of cancer, as predicted by agar plate assay run on each patient's tumor to select chemotherapy. Massive tumor usually dooms available chemotherapy and immunotherapy. Therefore, tumors are first reduced maximally by excision, cautery, direct intratumor injection of chemotherapy or cryogenic congelation before parenteral chemotherapy is given, followed by autogenous vaccine and maintenance chemotherapy.

Agar plate assays used to select clinical cancer chemotherapy have also predicted accurately known chemotherapy results with animal tumors, for a wide variety of drugs, such as 5-FU, hydrocortisone, Thiotepa, Mitomycin C, Actinomycin D and sulfhydryl inhibitors. Such agreement of in vitro test results with known in vivo data is deemed essential to clinical application of drug sensitivity tests. Because of the accuracy of the tests, they are used to select the drug or combination of drugs most active against the tumor cells of each patient treated. Individualized chemotherapy is given both to patients massively involved with cancer and to patients receiving prophylactic adjuvant chemotherapy and immunotherapy after potentially curative surgery, such as radical mastectomy for breast cancer.

New Sulfhydryl Inhibitors for Coordinated Surgical-Chemical Therapy of Cancer. F. E. KNOCK: *145th Meeting, American Chemical Society, Sept. 1963, 0-23.*

Residual (or non-histone) protein bound to DNA contains sulfhydryl groups and is essential for maintaining the structure of chromosomes, as is DNA. Therefore, potent sulfhydryl inhibitors are being studied to attack protein associated with DNA, alone and in combination with drugs aimed at DNA, such as 5-FU and azauridine. For replacement of arsenoso sulfhydryl inhibitors, iodoacyl, acrylo and methacrylo types of sulfhydryl inhibitors have been synthesized, along with potent, long acting local anesthetics for surgical use with painful sulfhydryl inhibitors.

Because high molecular weight mandelamide anesthetics show promise for clinical use with sulfhydryl inhibitors, substituted mandelamides containing iodoacetyl groups have been prepared, as well as phenolic esters linked to iodoacetyl groups, for unstable bonding between sulfhydryl inhibitor and solubilizing moieties. In tests on human and animal cancers,

a variety of these new iodoacyl, acrylo and methacrylo derivatives show marked activity, frequently exceeding that of oxophenarsine (mapharsen) and frequently potentiated by active mandelamide anesthetics, such as AK-33 or N-(4-biphenyl)-N-(2-diethylaminoethyl) mandelamide hydrochloride.

For histochemical studies of attack by sulfhydryl inhibitors on nuclear protein, Arsenic Orange dyes have been synthesized. Most useful has been AOK-1 or N-(4-(4-arsenosobenzeneazo)-1-naphthyl)ethylene diamine.

Right Ventricular Aneurysm; A Review of the Literature and Report of a Case of Successful Repair With the Aid of Temporary Cardiopulmonary By-pass. H. C. STANSEL, JR., O. C. JULIAN, and W. S. DYE: *Journal of Thoracic and Cardiovascular Surgery*, Vol. 46, No. 1, pp. 66-76, July, 1963.

A case report is presented of a right ventricular aneurysm secondary to a bullet wound of the chest 43 years prior to admission to the hospital. A review of the literature is included and emphasizes the rare occurrence of this lesion.

The aneurysm of the right ventricle was verified with angiocardiographic studies prior to surgery, and was removed with the aid of a temporary cardiopulmonary by-pass. The patient made an uneventful recovery.

Right ventricular aneurysm is rarely secondary to atherosclerosis. A rare cause may be trauma, such as in the case reported. Of more importance clinically is the development of a right ventricular aneurysm secondary to surgical cardiectomy, and a number of these cases have been reported.

This case reported was believed to be the first right ventricular aneurysm successfully repaired with the aid of temporary cardiopulmonary by-pass.

Malignant Melanomas of The Skin of The Head and Neck. H. W. SOUTHWICK, D. P. SLAUGHTER, J. F. HINCAMP: *American Journal of Surgery*, Vol. 106, No. 5, November, 1963.

The authors in a previous review found that there was a distinct improvement in salvage when an elective regional node dissection was performed as part of the primary treatment of malignant melanoma in general. Subsequent examination of the statistical material suggested that while this was true for most anatomic sites of origin of the disease, those lesions arising in the skin of the head and neck might have a sufficiently different natural history as to validate a less aggressive primary approach to the problem. Fifty-eight patients with malignant melanoma of the head and neck were reviewed; 38 patients formed the definitive five-year treatment group. The over-all survival five years free of disease was 39.5 per cent. In contrast with the larger group, however, there was no significant improvement in salvage in those patients who had an elective node dissection over those patients who had a therapeutic node dissection if the primary tumor had been controlled. On the other hand, no patient who had a recurrent primary tumor and clinical evidence of regional node disease survived. It would seem that that crux of treatment success with this group of patients is the primary control of the original lesion. This can be accomplished only by wide surgical excision generally accompanied by skin graft. Elective neck dissections are reserved for patients in whom such dissections can be performed easily in anatomic continuity or when the primary tumor is recurrent and the prognosis is consequently so poor.

Multilocular Cysts of the Kidney in Children. E. A. DAINKO, W. R. DAMMERS and S. G. ECONOMOU: *The Journal of Pediatrics*, Vol. 63, No. 2, August, 1963.

A case of a unilateral multilocular cyst of the kidney in a 22-month-old child is presented. Multilocular cysts of the kidney are rare, this case being the tenth in the pediatric age group reported in the literature. Multilocular cysts of the kidney are differentiated from multicystic disease, polycystic disease, and simple cyst of the kidney. A set of criteria for categorizing multilocular cysts is reiterated. Some proposed theories of etiology of renal cysts are mentioned. Multilocular cysts of the kidney most commonly present as abdominal

masses found on a routine examination and are most often misdiagnosed as Wilms's tumors. Hematuria is seen less frequently. The treatment is nephrectomy or simple resection where the opposite kidney is absent or insufficient.

Recognition and Treatment of Acute Vascular Injuries Associated With Orthopedic Problems. W. S. DYE: *Chicago Medicine*, Vol. 66, No. 22, 913-914, November, 1963.

The problem of acute injuries to arteries and veins associated with orthopedic problems is reviewed. Contusion of an artery is emphasized as the most important clinical entity because of the frequent absence of injury to soft tissue and bone. Blunt trauma, such as a bumper injury, is a common cause. Emphasis is placed on exploration of the artery if there is any question of arterial injury. Resection of the contused segment and interposition of a saphenous vein autogenous graft is the treatment of choice.

Most injuries of arteries associated with fractures occur adjacent to joints such as a supracondylar fracture of the humerus or femur. If pulses are not restored after reduction of fracture, serious consideration should be given to exploration of the artery to repair possible damage. Simultaneous internal fixation of the fracture may be important to perpetuate the permanency of the vascular repair.

Fasciotomy of an extremely swollen extremity secondary to trauma may be necessary to prevent Volkmann's contracture.

An important group of patients are those in which the trauma may not result in any injury to the artery, but continuing reflex vasospasm may produce a so-called post-traumatic sympathetic dystrophy. Because of the minor nature of the injury, these patients may be diagnosed as malingerers. Appropriate sympathetic nerve blocks will prove diagnostic as well as curative in some instances. Sympathectomy may be necessary.



TABLE OF CONTENTS

<i>page</i>	
46.....	Automobile Accidents and Laryngeal and Upper Tracheal Trauma PAUL H. HOLINGER, M.D. AND JOHN W. CURTIN, M.D.
53.....	Red Cell Survival Studies EARL T. ANDERSON, M.D. AND FRANK E. TROBAUGH, JR., M.D.
60.....	An Experience with Coronary Arteriography GUY R. MATTHEW, M.D.
68.....	Cytomegalic Inclusion Disease—Review Article DONALD HENSON, M.D.
78.....	Abstracts and Reviews

AUTOMOBILE ACCIDENTS AND LARYNGEAL AND UPPER TRACHEAL TRAUMA

PAUL H. HOLINGER, M.D.¹

JOHN W. CURTIN, M.D.²

The increasing frequency of laryngeal injury is, in a large measure, due to automobile accidents. In a previous summary of our experience with laryngeal trauma and its complications, 43 of 82 laryngeal fractures were reported as having occurred in transportation vehicles, 32 of them in automobiles.¹ Others report a similar high incidence.² Thirty-three of the 82 patients in our series had required surgery for restoration of function of whom 13 had required total laryngeal or cervical tracheal reconstruction.

The larynx is protected from frontal trauma to some extent by the chin and chest; however, baseball bats, clotheslines, knives of cutthroat artists, the dashboard and steering wheels of automobiles and the edges of heavy objects being carried by persons who stumble and fall on them, find a way of getting between the chin and the chest to slash the larynx or crush it against the cervical spine. In addition to these compressions and lacerations, evulsions and hemorrhage into the tissues result in obliteration of the airway and destruction of the phonating function of the larynx. In the automobile accidents that are accounting for so many of these problems, it is usually the front seat passenger who is in a car involved in a head-on collision with another vehicle, or in one striking an abutment. Occasionally, the driver is the victim if his neck should strike the steering wheel. The individual is thrown forward with the

head and neck extended, and it is in this position that the exposed neck strikes the dashboard or the steering wheel. The impact results in a compression fracture of the thyroid, cricoid or tracheal cartilages. Only one of the patients in our series was wearing a seatbelt at the time of the accident.

In these external injuries of the larynx, the pathology may vary from simple hematomas with slight contusions or minimal lacerations, to total compound fractures of the thyroid and cricoid cartilages or evulsions and separation of the trachea from the cricoid cartilage. In severe trauma, the arytenoids are completely dislocated and forced anteriorly (Fig. 1) and extensive emphysema, hemoptysis, jagged tears and hematomas develop. Profound respiratory obstruction obscures the normal anatomical landmarks, sometimes making immediate evaluation impossible. In such cases the laryngeal aspect is generally but a part of a multiple injury accident and an emergency tracheotomy is imperative for the immediate care of the associated injuries as well as to make it possible to provide the proper management of the crushed larynx itself.

Since 1959 when the previous article describing our experience with this problem was published, 34 patients with severe laryngeal or tracheal trauma have

From the Departments of Otolaryngology and Plastic Surgery, Division of Surgery

¹ Attending Otolaryngologist (Bronchoesophagology), Presbyterian-St. Luke's Hospital
Professor of Bronchoesophagology, Otolaryngology Department, University of Illinois, College of Medicine

² Associate Attending Surgeon, Presbyterian-St. Luke's Hospital
Clinical Associate Professor of Surgery, University of Illinois, College of Medicine

been seen. In 28 the injury had been incurred in an automobile accident. In only two instances did we see the patient in the immediate post-accident period when primary repair was possible. All others were seen from two months to twenty years after the accident when deforming cicatricial changes had seriously impaired or totally obstructed the airway. (Fig. 2) Nineteen were males, 15 females, their ages ranging from 7 to 73 years; the age distribution by decades is indicated in Table No. 1. Twenty-eight of the 34 patients had required a tracheotomy. Three were seen only in consultation, 16 were managed by endoscopic procedures such as removal of granulation tissues, endolaryngeal arytenoidectomy when

TABLE NUMBER 1
Age incidence of 34 patients with severe laryngeal trauma. (28 incurred in automobile accidents)

AGE	
0- 9 years.....	1
10-19 years.....	6
20-29 years.....	9
30-39 years.....	8
40-49 years.....	3
50-59 years.....	3
60-69 years.....	3
over 70 years.....	1

dislocated arytenoids were primarily responsible for airway obstruction, dilations or the insertion of internal laryngeal splints. However, 15 of the 31 pa-

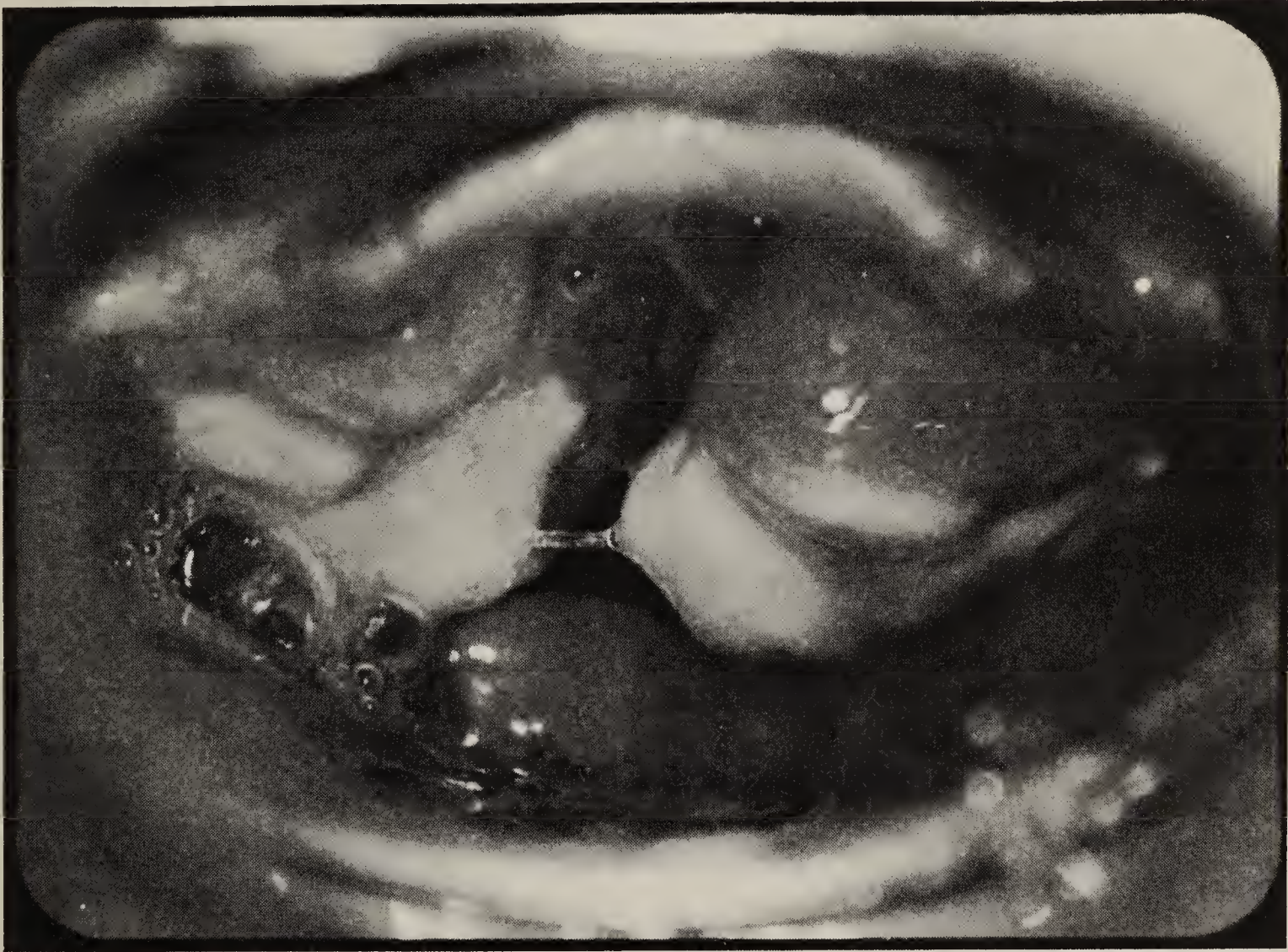


Fig. 1.—Mirror view of the larynx 48 hours following an automobile accident. The thyroid and cricoid cartilages were crushed against the cervical spine by the dashboard. The arytenoids have been dislocated anteriorly, the cords are flaccid, edematous, covered with exudate and bow inward and the airway is obliterated.

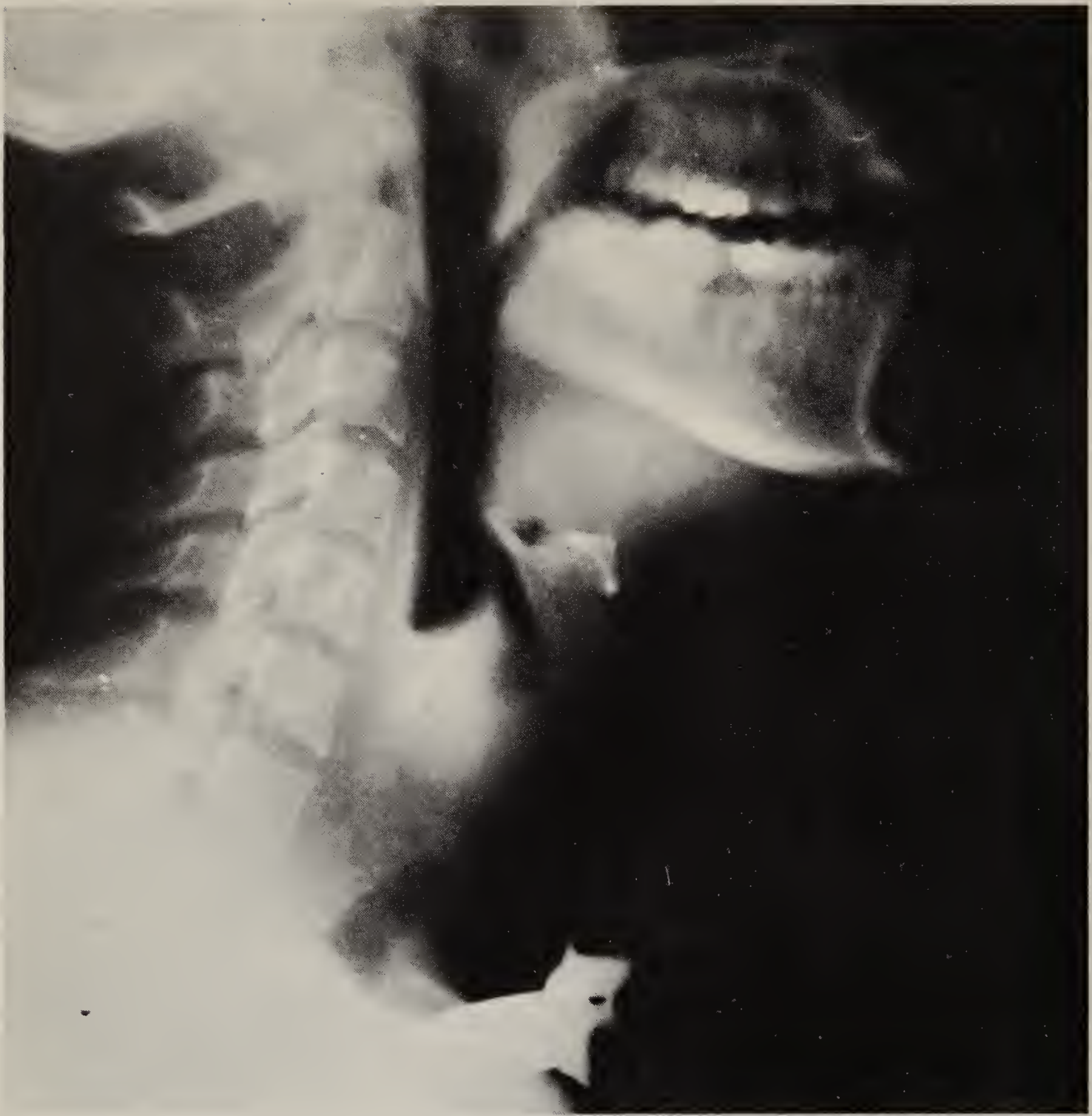


Fig. 2.—Total atresia of the larynx following automobile accident injury.

tients treated required extensive external laryngeal or cervical trachea reconstruction because stenosis or atresia was so complete that endoscopic means were inadequate to re-establish and maintain an airway. This discussion deals primarily with this group in whom indica-

tions and techniques of reconstruction were guided by the extent of the injury and the subsequent deforming scar tissue contractures. Management of the acute injury to avoid these serious complications is considered in the discussion.

TECHNIQUE

In laryngeal or cervical tracheal injuries that have resulted in chronic airway stenosis or atresia, consideration is given to reconstructive procedures only after all evidence of inflammation and perichondritis has disappeared and ap-

propriate measures have been undertaken to eliminate any residue of infection. As accurate a diagnosis as possible of the degree of cartilage and soft tissue involvement must be made. Mirror and direct laryngoscopy, tomographic roent-

genograms and antegrade and retrograde x-ray studies with contrast media are carried out prior to surgical exploration. The type of surgical repair will depend on the extent and magnitude of the cicatricial laryngeal stenosis. If success is to be accomplished the lining must include complete epithelial continuity. The danger of infection and failure of repair will continue as long as any raw surface remains.

A continuous lumen is first established by proceeding through a midline neck incision. The trachea and larynx are opened from a point well below the stenosis upward to the pharynx. If the trachea alone is involved and the airway through the cricoid and thyroid cartilages is normal, or at least adequate, the larynx, *per se*, is not disturbed. Lowering of the tracheostomy opening becomes necessary in most cases to avoid the involved pathology. In cases of complete atresia all obliterating cicatricial tissues such as scar, granulations and cartilaginous protrusions must be removed. A

soft rubber nasogastric tube within the lumen of the esophagus will aid in preventing accidental openings into this intimately attached structure. When laryngeal involvement was severe, major obstructions in this series of patients were often found to be caused by the arytenoid cartilages which had been dislocated and fixed anteriorly. Removal of the arytenoids in this situation usually resulted in a marked improvement of the airway lumen.

After the establishment of an adequate lumen, those portions of the interior of the larynx or trachea which were denuded, a lining must be provided and we have preferred the use of the split-thickness skin graft. A hairless graft of intermediate thickness is with its exposed dermal surface outward wrapped around a pre-formed piece of sterile foam rubber. (Fig. 3) Once meticulous hemostasis has been obtained, the stent graft is inserted into the lumen of the larynx and trachea. The tissues of the airway are closed over the graft with interrupted fine catgut and

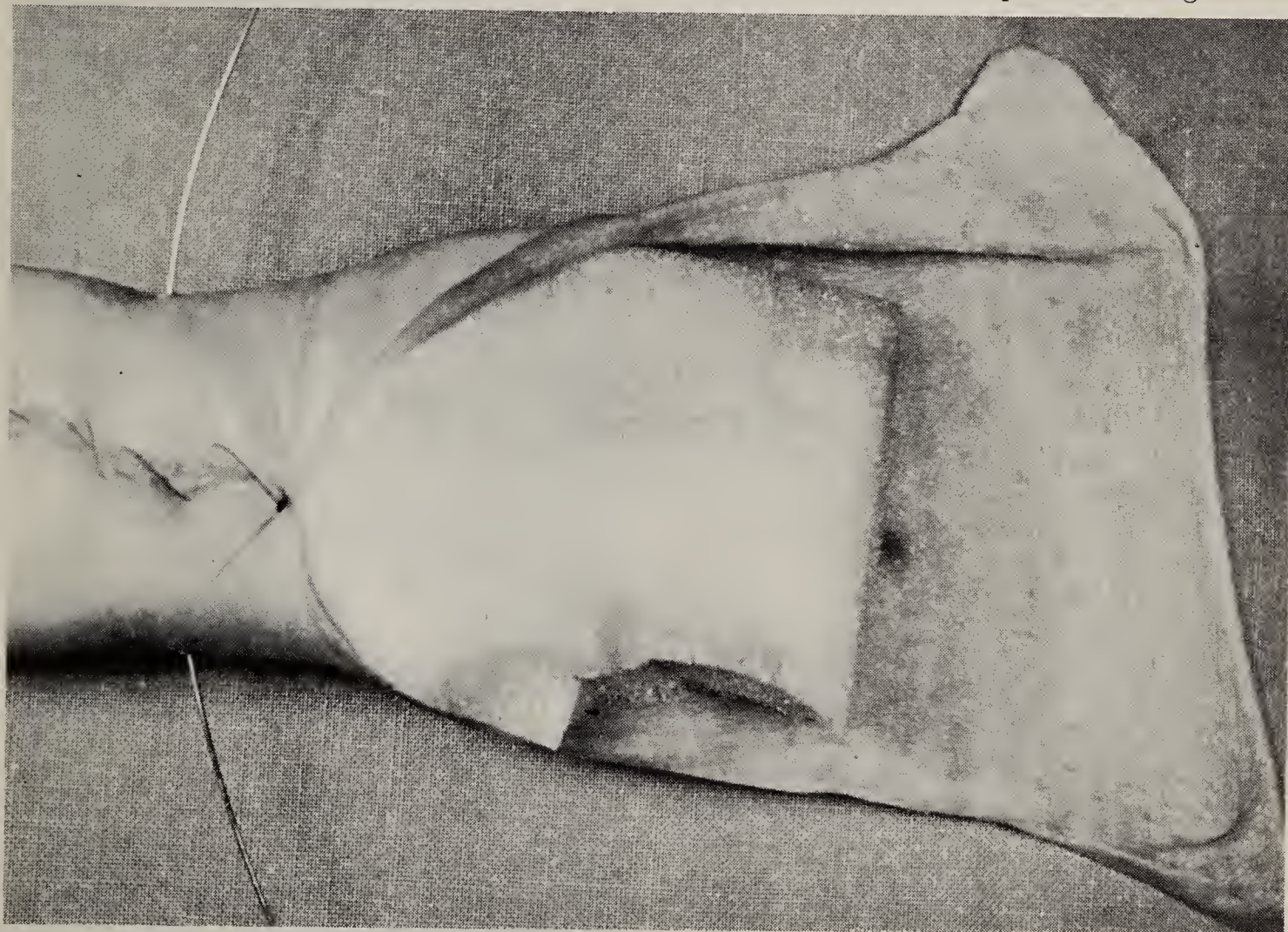


Fig. 3.—Stent graft of the larynx. The split-thickness skin graft is wrapped around the pre-formed piece of foam rubber. The steel wire passes through the lateral structures of the neck to hold the stent in place.

nylon sutures. The sausage-shaped graft and its encased foam rubber stent are held in place by a piece of #25 stainless steel wire. This passes through the graft and stent as well as the lateral structures of the neck preventing dislodgement upward into the pharynx or downward into the trachea or bronchus.

Specific, detailed attention to all phases of postoperative care is most essential. The patient's head is maintained in a rigid position during the immediate ten day postoperative period. Feedings are given by nasogastric or intravenous methods to avoid the motion of swallowing.

On the tenth to the twelfth day, the foam rubber is removed from the interior of the larynx by direct laryngoscopy, the skin graft lining is observed and a polyethylene tube, closed at both ends, is inserted into the larynx and fixed externally by a heavy braided silk cord brought out through the tracheostomy opening. (Fig. 4) This polyethylene splint is changed at intervals of two months; its purpose is to maintain the lumen size for as long as a year to avoid contracture of the graft and a recurrence of the constriction.

In nine months to a year, when it is demonstrated that the polyethylene tubing can be left out permanently and the airway is adequate, the tracheotomy tube is removed and the tracheostomy closed. At this time vocal therapy is begun by a qualified speech therapist in the Division of Speech. This is an exceedingly important part of the rehabilitation attempt to restore satisfactory voice function.



Fig. 4.—Polyethylene tube in the larynx maintaining a lumen as the cartilages heal in a position which will maintain the airway.

DISCUSSION

In view of the prolonged treatment necessary when stenosis has developed, every effort should be made to stress the basic principles of initial diagnosis and treatment at the time of the accident and immediately thereafter. Fractures of the larynx, just as fractures elsewhere in the body, require adherence to the basic principles of all trauma surgery; namely, restore the parts to as nearly a normal

position as possible and splint them in a position of function. This is just as true of the larynx as it is of other structures but, unfortunately, delay is too often encountered. The reasons for this delay are generally that the patient has so many other injuries that once the tracheotomy has established the airway, the laryngeal injury itself and its management are overlooked. Early recognition of the ex-

tent of damage is essential and this may be done by both x-ray studies and direct laryngoscopy. If fractures of the cartilaginous supporting structures, namely, the thyroid and cricoid cartilages are evident at the time of injury, immediate plans for restoration of the airway should be made. As soon after the accident as possible and the patient's condition permits, an internal splint consisting of a polyethylene tube may be introduced into the larynx. This serves to restore the cartilages to their position of function, maintaining a lumen. The polyethylene tube is fixed externally and should extend above and below the points of fracture. (Fig. 5) Sufficient rigidity to insure the support of the airway lumen should be obtained in six weeks to two months at which time the internal splint may be removed and the tracheostomy closed. In some cases an open reduction bringing the portions of cartilage into alignment may assist in restoring at least the framework of the airway.

Management of the two patients in this series seen in the immediate post-

accident period resulted in prompt and permanent restoration of the airway—one through external repair of a tracheal compression fracture and one through an immediate intralaryngeal re-alignment of compressed thyroid and cricoid cartilage fractures supported by an intralaryngeal splint. This may be compared to the accepted routine of early treatment of a severe compression fracture of the nose.

As mentioned above, 16 patients were managed by endoscopic procedures alone—dilations, removal of granulomas, intralaryngeal arytenoidectomy. Four responded to endoscopic dilations or removal of obstructing granulations without tracheotomy. Twelve were tracheotomized, of whom eight have been extubated and two are under treatment; the other two will require external reconstruction.

Of the fifteen patients requiring the extensive surgical reconstructive procedure described above, eight have been extubated and seven are still under treatment. The ultimate goals are restoration of the airway through the mouth and the development of a serviceable voice. The prolonged and repeated hospitalization, the many surgical procedures and the months of tracheotomy care represented in this group indicate the need for a more aggressive approach at the time of the accident. In all 15, the fractured cartilages had healed as they had been compressed at the time of the accident, obliterating the lumen of the airway.



Fig. 5.—Direct laryngoscopic view of a skin graft in the larynx following total laryngeal reconstruction for laryngeal atresia. The laryngeal injury resulted from an automobile head-on collision in which the patient was the front seat passenger. Seatbelts had not been used.

SUMMARY

Laryngeal and cervical tracheal accidents are often sustained in accidents involving transportation—automobiles, buses, trains, airplanes, tractors, bicycles and sleds. The laryngeal injuries are usually one part of a multiple injury accident. Physical violence is next in frequency as a cause of laryngeal trauma; this includes “strong arm” and cut-throat injuries as well as sports accidents, in boxing or being struck by a baseball bat. Laryngeal accidents in industry include striking protruding pipes, stumbling and falling on the edge of a heavy object being carried, or looking through horizontal elevator doors—the doors or the elevator closing on the neck!

Treatment of minimal injuries consists of hot packs, voice rest (silence) and steam inhalations, with voice rehabilitation to help restore this important laryngeal function after the acute manifestations have subsided.

Severe injuries to the larynx require low tracheotomy to establish the airway. Then, as soon as the patient's general condition will permit, broken cartilages must be replaced and held in position by an intralaryngeal splint or mold to insure an adequate airway. If manipulation perorally cannot be accomplished because of an associated fractured jaw, this must be performed through the tracheostomy or by external, open reduction.

Failure or inadequate early management results in chronic laryngeal stenosis and the need of a permanent tracheostomy. Treatment of this complication requires reconstruction of the laryngeal lumen through the removal of scar and deformed cartilage and the lining of the interior of the larynx with a split thickness skin graft. This long and tedious process can be avoided by early reduction and fixation of the laryngeal fractures.



REFERENCES

1. Holinger, P. H. and Johnston, K. C. Laryngeal Trauma and its Complications. *Am. Journal of Surg.*, 97:4:513-517, 1959.
2. Fitz-Hugh, G. S., et al. Injuries of Larynx and Cervical Trachea. *Ann. Otol. Rhinol. Laryngol.* 71:419, 1963.

RED CELL SURVIVAL STUDIES

EARL T. ANDERSON, M.D.¹

FRANK E. TROBAUGH, JR., M.D.²

The erythrocyte is a highly specialized cell without a nucleus, incapable of reproduction and probably with limited capacity of self repair. There is constant production and destruction of red cells and the excretion of bile pigment is but one evidence of the continuity of this process and a reminder that approximately 1% of the red cells of the body are destroyed daily. The many factors concerned in the ageing of the red blood cells are beyond the scope of this presentation, but there is considerable evidence that erythrocytic destruction is normally a function of cell age while there may be in addition, a variable degree of random destruction unrelated to cell age. The manner and sites of *normal destruction* of red blood cells are unknown, but it seems that hemolysis and phagocytosis of red cells play but little role. The last comprehensive evaluation of this problem was presented in 1923 by Rous and Robertson who proposed that the chief means of senescent red cell destruction was by fragmentation as the red cells were buffeted about and became distorted in the circulation. This fragmentation continued until finally a fine hemoglobin dust was formed which was removed by phagocytic cells of the reticulo-endothelial system.

The life span of red blood cells and sites of destruction are not only of basic and fundamental interest to us as physicians, but knowledge of them permits a greater precision of diagnosis thus providing the basis for a sound approach to therapy.

The red blood cell is probably the only mammalian cell whose life span can be satisfactorily determined in the intact animal. Throughout the years many methods of studying its life span have been

tried but many of these are now of only historical interest. The differential agglutination technique, the oldest of the valid methods for the measurement of the red cell life span, was first used in cattle in 1911 by Todd and White and in 1919 was applied to humans by Ashby. It has since come to be known as the "Ashby Technique." In this procedure compatible but immunologically identifiable blood is transfused into a recipient and periodically the donor red cells are enumerated following agglutination of the recipient's red cells by a specific anti-serum. For example, if type O red cells are given to a group A recipient the absence of isoantibodies to the donor cells allows them to survive normally. At intervals, samples of the recipient's blood are incubated with anti A serum resulting in agglutination of the recipient's group A cells. The unagglutinated type O donor cells are then counted. Unag-

From the Section of Hematology of the Department of Medicine
Supported by U.S.P.H.S. Grant C A 05429

¹ Adjunct Physician, Presbyterian-St. Luke's Hospital
Instructor in Medicine, University of Illinois College of Medicine

² Attending Physician, Director of Section of Hematology, Presbyterian-St. Luke's Hospital
Associate Professor of Medicine, University of Illinois College of Medicine

glutinated cells normally disappear from the circulation in a linear fashion in 100 to 120 days.

The Ashby Technique has provided great insight into red cell survival in health and disease and continues to serve as a frame of reference for other techniques. It has, however, several disadvantages: (1) Large volumes (0.5–1.0 liters) of blood must be transfused in order to obtain reasonable unagglutinated cell counts. (2) Before the transfusion, the recipient must be bled a volume equal to the amount of blood to be given in order that the “steady state” of the circulation is not disturbed. (3) The life span of the subject’s red cells cannot be measured in his own circulation. (4) The technique is laborious, time consuming and inherent errors are great. (5) Blood group limitations restrict its usefulness.

With the close of World War II, the age of nuclear medicine arrived and isotopic methods for studying red cell survival were proposed. As early as 1945, it was demonstrated that glycine labeled with the stable isotope N^{15} was incorporated into protoporphyrin, thus labeling hemoglobin and providing a method of measuring erythrocyte survival. Later, glycine labeled with the active isotope C^{14} was used to identify hemoglobin in the same manner. The use of N^{15} or C^{14} labeled glycine has the important advantage that it is metabolically incorporated into the hemoglobin of the red cell. However, several factors have limited the use of these techniques as diagnostic procedures: (1) The isotope must be incorporated into the red cell *in vivo*. (2) The technique of preparing the hemoglobin for counting is difficult. (3) The use of N^{15} requires a mass spectrometer, available in very few hospitals. (4) Only recently has equipment suitable for counting C^{14} been widely available. (5) The carbon and nitrogen released by red cell destruction are reutilized. Fe^{55} and Fe^{59} have also been used in the study of red cell life span but suffer from some of the same limitations as do the C^{14} and N^{15} techniques.

With the demonstration by Gray and Sterling in 1950 that sodium chromate penetrates and becomes fixed in the red cells, a new method of measuring red cell life span became possible. In principle, the hemoglobin of red cells incubated *in vitro* with sodium chromate 51 becomes labeled with the gamma ray emitting Cr^{51} . After reinjection of this labeled blood, it is a simple procedure to estimate the *in vivo* survival of red cells by counting the amount of Cr^{51} remaining in the peripheral blood. Cr^{51} has the advantages that it is firmly bound to the red cell, is non-toxic to the cell, is not reutilized and is readily detected and quantitated.

The technique may be summarized as follows: 16 ml. of the patient’s blood is incubated for 45 min. in 4 ml. of a special acid citrate dextrose solution* containing 100 microcuries of Cr^{51} as sodium chromate ($Na_2Cr^{51}O_4$). To remove any unbound chromium, the red cells are washed in saline three times, resuspended in saline and injected into the patient. Cr^{51} activity in each sample of whole blood is determined in a well type scintillation counter after saponin hemolysis. The counts obtained in a ten minute blood sample are used to determine the patient’s red cell mass and blood volume utilizing the dilution principle and the same sample serves as the base line value or 100% activity level in the survival studies. Blood samples are taken at appropriate time intervals until the level of Cr^{51} in the peripheral blood is 30–35% of the ten minute value. As Cr^{51} emits gamma rays, it is possible to detect the isotope in various body organs by use of external scintillation detectors. Thus, at the time that each blood sample is obtained, the amount of Cr^{51} and thus presumably the red cell content is determined by external body scans over the heart, liver and spleen. Counts over the heart are assumed to result from the Cr^{51}

*Supplied as A-C-D Solution by Abbott Laboratories. Each ml. contains Dextrose 13.2 mg., Sodium citrate 25.0 mg and Citric acid 8.0 mg.

content of the circulating blood. Counts over the spleen and liver result from their blood content plus sequestered red cells. Thus, by dividing the counts over the spleen or liver by the precordial counts, an organ:precordial ratio is obtained providing an index of red cell sequestration and destruction.

When the red cells of a normal subject are labeled with Cr^{51} and returned to the donor or infused into another normal recipient, the points plotted from the data obtained by serially sampling and assaying the peripheral blood radioactivity do not fall on a straight line as do the points derived from an Ashby test. (Fig. 1) The Cr^{51} survival time is the resultant of the loss of red cells by ageing *and* the loss or elution of Cr^{51} from the surviving red cells. The rate of Cr^{51} elution has been shown to be approximately 1% per day in the normal. This figure has been obtained by simultaneous measurement of red cell survival using Cr^{51} and another technique. At present there is no generally accepted method to correct for elution and it has become customary to present the uncorrected results. Nevertheless, the information derived from survival studies using Cr^{51} is so valuable and the technique so satisfactory and widely applicable that the method is deservedly popular.

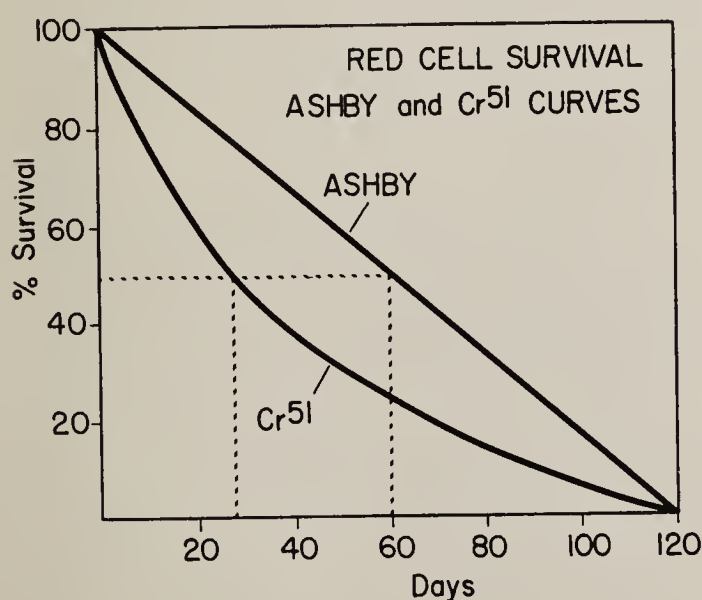


Fig. 1.—Comparison of erythrocyte survival as determined by differential agglutination (Ashby) and Cr^{51} techniques.

The presently available data indicate that the labeling chromium enters the cell, changes valency, and is firmly bound to hemoglobin. The data are conflicting about whether it is bound to the alpha or beta chain of hemoglobin. The small amount of chromium that does elute from the cells and the chromium freed by cell destruction are excreted in the urine and do not relabel cells to an appreciable extent. The time for the Cr^{51} activity to decrease to 50% ($T_{1/2}$) is customarily used as the endpoint in this procedure. Normal survival figures vary from laboratory to laboratory but in general the normal Cr^{51} red cell half time is 25–33 days.

Cr^{51} SURVIVAL IN HEMOLYTIC STATES

The sine qua non of hemolysis is the demonstration of a shortened red cell survival. Reticulocytosis, polychromasia of the red cells, hyperbilirubinemia and erythroid hyperplasia of the bone marrow are well known features of hemolytic anemias but are all indirect and secondary evidences of hemolysis. They provide, at best, poor quantitation of the degree of hemolysis and little or no insight into the mechanisms or sites of destruction. Measuring the Cr^{51} in the peripheral blood provides a good estimate of the degree of hemolysis and scanning over the liver and spleen provides evidence for red cell sequestration in these organs. In addition, by tagging both autologous and homologous cells it is possible to determine whether the defect resulting in hemolysis resides in the patient's cells or in the plasma; i.e. whether the defect is intracellular or extracellular. Following the demonstration and quantitation of hemolysis by tagging the patient's own cells (autologous), it may be of value to follow the survival of homologous (donor) cells in the same patient. If these homologous cells survive normally, the assumption may be made that the defect is inherent in the patient's cells. If, however, the survival of donor cells is also shortened it follows

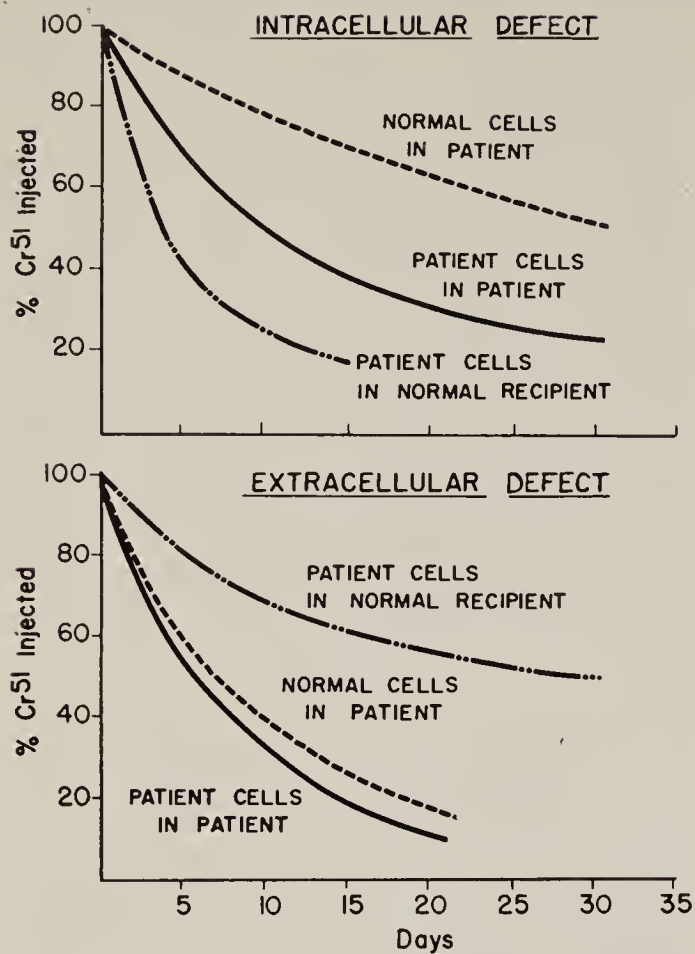


Fig. 2.—Survival of Cr^{51} labelled red cells in presence of intracellular and extracellular defects.

that the defect is in the patient's plasma. (Fig. 2) These assumptions can be tested by following the survival of the patient's cells in a normal recipient. If there is a shortened survival the defect is intracellular while a normal survival in the recipient suggests an extracellular defect.

A. Intracellular Defects

1. Hemoglobinopathies: A shortened red cell survival is seen in those diseases in which normal hemoglobin A has been replaced entirely by abnormal hemoglobin(s). The most common of these is sickle cell anemia in which the red cells contain only hemoglobin S. Such red cells have a markedly shortened half time, usually from 1 to 8 days. (Fig. 3) The red cells of patients with sickle cell trait, which contain more than 50% hemoglobin A, usually show a normal survival. In like manner, red cells in patients with other hemoglobinopathies

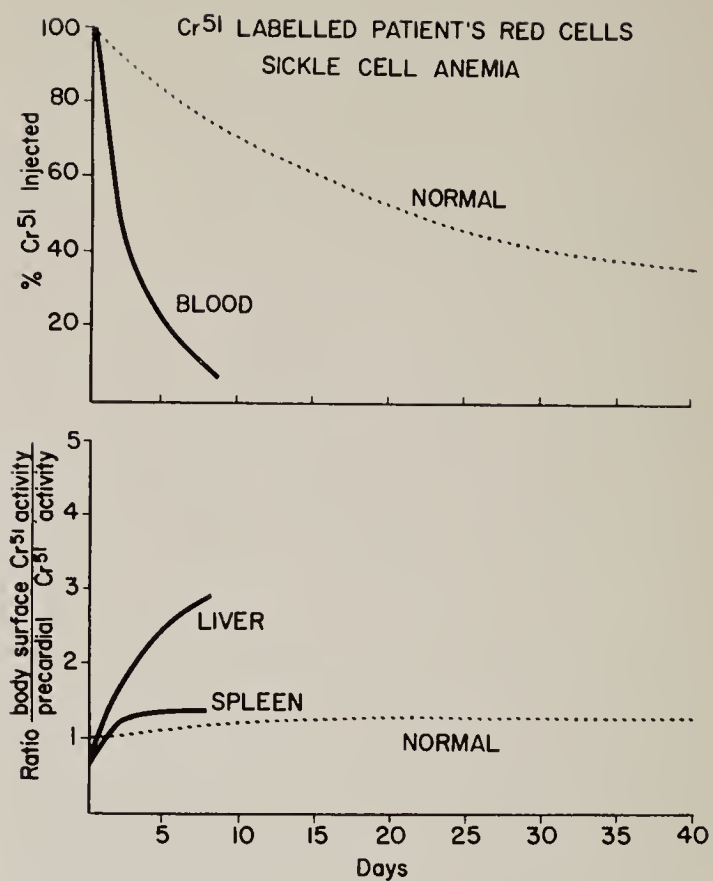


Fig. 3.— Cr^{51} red cell survival in sickle cell anemia.

(S-C, C-C, etc.), show a shortened survival but to a lesser degree than that seen in S-S disease. These abnormal red cells show essentially the same degree of shortened survival in a normal recipient as they do in the patient. Conversely, normal donor red cells survive normally in the patient.

It should be remembered that a patient with the congenital hemolysis of a hemoglobinopathy may develop an acquired hemolysis to add to his difficulty. Under such circumstances, normal donor cells also show a shortened survival and the patient's own cells show an even shorter survival than they do regularly. This probably accounts for the occasional hemolytic crisis seen in a child with S-S disease, a disease in which there tends to be a steady hemolysis unassociated with crises.

2. Thalassemia: In thalassemia major

or Cooley's anemia there is similarly a markedly diminished survival of autologous red cells and normal survival of donor cells. In thalassemia minor, the heterozygous state, the survival is normal or moderately shortened.

3. Congenital Spherocytic Disease: Congenital spherocytic anemia is another disease in which the defect is intracellular. As is the case in the other intracellular defects, the affected red cells show shortened survival in the patient or in a normal recipient but a distinguishing feature of this disease is that the patient's red cells survive *normally* in a splenectomized recipient. External organ scanning shows a marked increase in radioactivity over the spleen, confirming the spleen's role in destroying the affected red cells. Following splenectomy, the red cell survival returns essentially to

normal and so does the patient's health, even though the erythrocyte defect persists. (Fig. 4)

4. Non-spherocytic hemolytic anemia: Other disease states characterized by intracellular defects leading to decreased survival times include the non-spherocytic hemolytic anemias. These comprise a heterogeneous group of familial anemias in which the red cells may be large, oval or normal in appearance. They show no spherocytosis and no alteration in osmotic fragility. These states may result from enzyme deficiencies some of which, such as deficiencies of glucose-6-phosphate dehydrogenase and pyruvate kinase, have been entirely defined. The glucose-6-phosphate dehydrogenase deficient states are aggravated by exposure to certain chemicals such as primaquine, sulfonamides, quinine and quinidine.

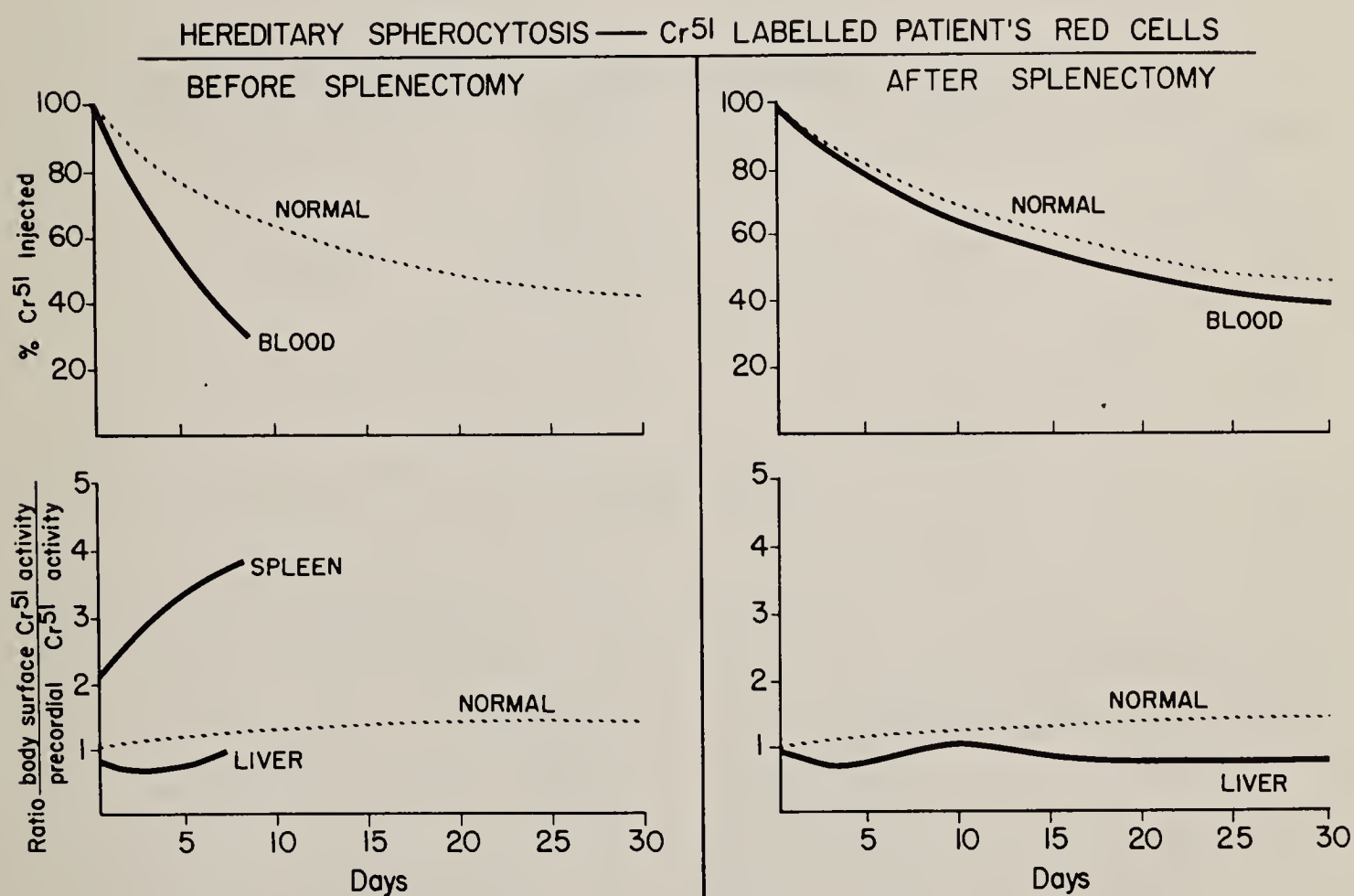


Fig. 4.—Hereditary Spherocytosis: Comparison of the Survival of Cr⁵¹ Labelled Autologous Red Cells Before and After Splenectomy.

5. Deficiency States: Anemias resulting from a deficiency of vitamin B₁₂, folic acid or iron, although not primarily hemolytic anemias, are characterized by some apparent shortening of the red cell life span. In these, the principle defect is one of erythrocyte production as occasioned by the responsible deficiency.

It should be noted that, except for the nutritional deficiencies, all of the above are hereditary defects or inborn errors of metabolism and are associated with alterations in the hemoglobin molecule, in porphyrin synthesis or in intracellular energy metabolism.

B. Extracellular Defects

In contrast to the intracellular defects, the extracellular or plasma defects are usually not hereditary but acquired. Some of these are associated with demonstrable antibodies and constitute the autoimmune hemolytic anemias. Others show no antibodies and the hemolysis

results from non-immunologic mechanisms such as direct chemical toxicity, parasitism, etc. All of these hemolytic anemias are characterized by shortened survival of the patient's *and* donor cells in the patient's circulation and *normal* survival of the patient's cells in a normal recipient. (Fig. 5)

The autoimmune hemolytic anemias may be primary (idiopathic) or secondary (symptomatic) to another illness. In the primary or idiopathic cases, there is no demonstrable causative or underlying disease. The symptomatic autoimmune anemias result from many diseases, some of the more common being chronic lymphocytic leukemia, lymphocytic lymphomas, systemic lupus erythematosus, viral pneumonia and infectious mononucleosis.

In all of the autoimmune hemolytic anemias, whether primary or secondary, there is an increased uptake of Cr⁵¹ over the liver and/or spleen, but it is not

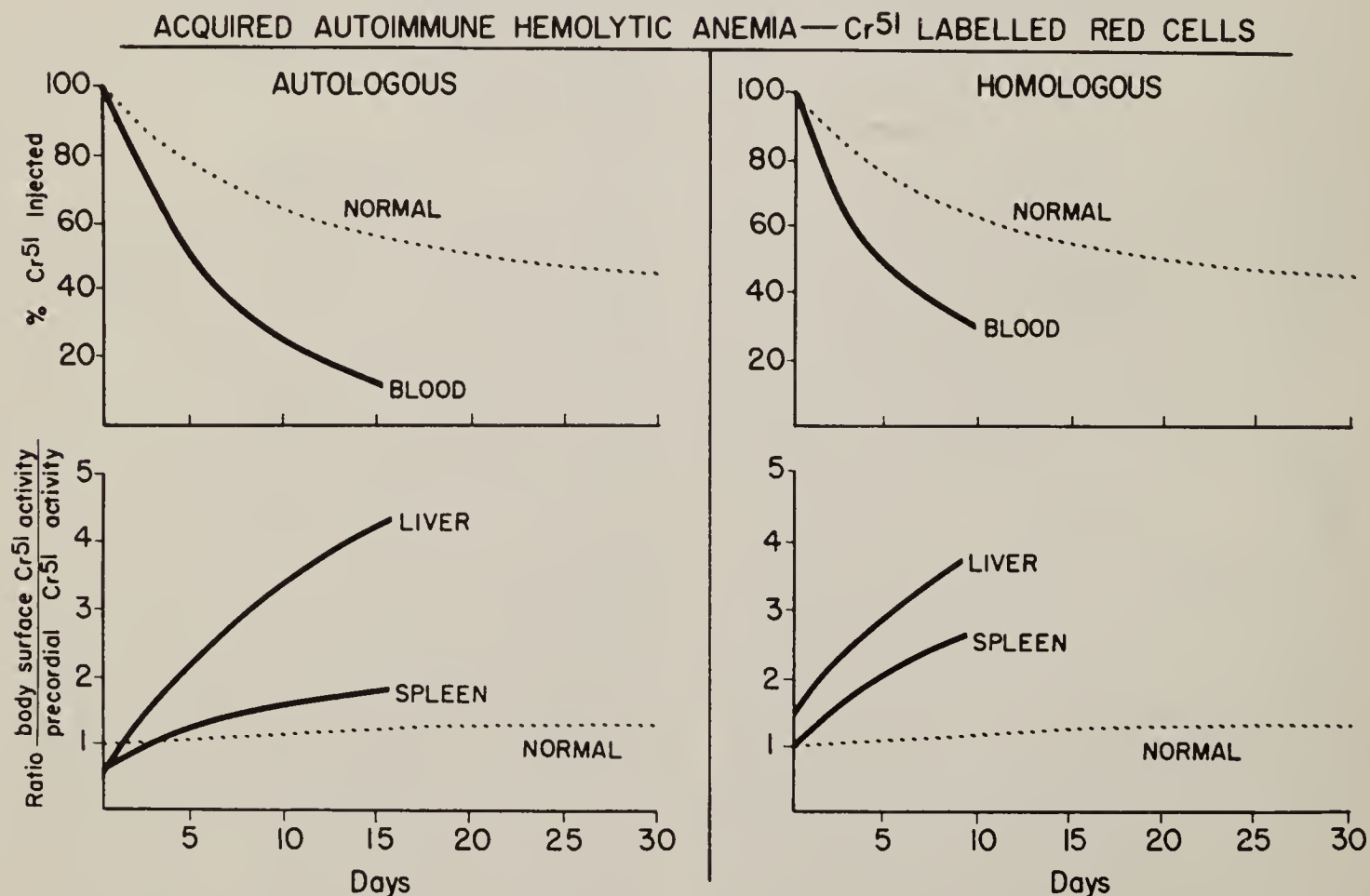


Fig. 5.—Chromium⁵¹ red cell survival and organ radioactivity in patients with acquired autoimmune hemolytic anemia.

possible to predict which organ will be most responsible for red cell sequestration and destruction. The work of Jandl and others suggests that there is a relationship between the type of antibody present and the site of destruction. In the presence of incomplete, or sensitizing antibodies (such as those of the Rh system) the Cr⁵¹ labeled red cells disappear in a logarithmic fashion and there is a striking increase in radioactivity over the spleen. When the responsible agent is a complete antibody (such as is found in the ABO system) the destruction of Cr⁵¹ labeled red cells is more rapid and the greatest radioactivity is over the liver. Although these investigations are of much interest, much more work is needed to identify the nature of the various antibodies and the relationship between the type of antibody and the site and mechanism of red cell destruction. In the present state of knowledge, the organ uptake of Cr⁵¹ provides the best prediction of possible benefit from splenectomy.

Many acquired hemolytic states are not accompanied by demonstrable antibodies. These include three principal groups:

1. Those caused by chemicals which have a direct toxic effect on normal erythrocytes. These include heavy metals, arsine and phenylhydrazine. Water is included in this category because, when it is introduced into the blood stream, as has happened occasionally following transurethral prostatic resection, it may produce significant hemolysis.

2. Those caused by infectious agents. The parasites of malaria and bartonella may directly destroy red cells, while bacteria result in red cell destruction through the elaboration of toxins.

3. Those caused by unknown mechanisms. There remains the occasional patient in which there is a variable degree of extracellular hemolysis associated with any one of a wide spectrum of illnesses. Such have been seen rarely in a variety of malignancies, acute infections, acute renal failure and chronic inflammatory diseases such as rheumatic fever. These have been little studied, the mechanisms are unknown and no pattern of excessive sequestration by any part of the reticulo-endothelial system has been demonstrated.

REFERENCES

1. Ashby, W.: Determination of Length of Life of Transfusion Blood Corpuscles in Man. *J. Exper. Med.*, 29:267, 1919.
2. Ashby, W.: Span of Life of Red Blood Cell. *Blood*, 3:486, 1948.
3. Berlin, N. I., Waldman, T. A. and Weissman, S. M.: Life Span of Red Blood Cell. *Physiological Reviews*, 39:577, 1959.
4. Berlin, N. I. and Cline, M. J.: The Red Cell Chromium Elution Ratio in Patients with Some Hematologic Diseases. *Blood*, 21:63, 1963.
5. Dacie, J. V.: *The Hemolytic Anemias; Part II—The Autoimmune Hemolytic Anemias*, Grune and Stratton, New York, 1962.
6. Ebaugh, F. G., Jr., Emerson, C. P. and Ross, J. A.: Use of Radioactive Chromium⁵¹ as Erythrocyte Tagging Agent for Determination of Red Cell Survival in Vivo. *J. Clin. Invest.*, 32:1260, 1953.
7. Gray, S. J. and Sterling, K.: Tagging of Red Cells and Plasma Proteins with Radioactive Chromium. *J. Clin. Invest.*, 29:1604, 1950.
8. Harris, J. W.: *The Red Cell*, Harvard University Press, Cambridge, 1963.
9. Jandl, J. H., Greenberg, M. S., Yonemoto, R. H. and Castle, W. B.: Clinical Determination of Site of Red Cell Sequestration in Hemolytic Anemias. *J. Clin. Invest.*, 35:842, 1956.
10. Jandl, J. H., Jones, A. R. and Castle, W. B.: The Destruction of Red Cells by Antibodies in Man. Observations on the Sequestration and Lysis of Red Cells Altered by Immune Mechanisms. *J. Clin. Invest.*, 36:1428, 1957.
11. Wintrobe, M. M.: *Clinical Hematology*, Lea and Febiger, Philadelphia, 1961.

AN EXPERIENCE WITH CORONARY ARTERIOGRAPHY

GUY R. MATTHEW, M.D.¹

The purpose of this paper is to outline the technique of *in vivo* coronary artery opacification developed at the Thoracic Clinics of the Karolinska Hospital, Stockholm, Sweden by Docent Björn Nordenström and his colleagues, and to describe a brief and unsuccessful animal experiment designed to enhance the radiographic appearance of the myocardiographic phase of that examination.

Coronary arteriography was initially reported in 1933,^{1,2} with Radner³ given credit in 1945 for initial demonstration of the coronary arteries in the intact human.⁴ In general, the earlier attempts at this procedure were hampered by rapid disappearance of the contrast material away from the point of deposition, and it was realized that contrast injections during conventional angiographic procedures did not result in optimum visualization of the coronary arteries. More recently, several methods have been developed for increasing the percentage of contrast flow into the coronary system, while also reducing total volume of the more tolerable contrast materials injected. These methods range from direct coronary injection to various physical, chemical and electrical procedures designed to deliver maximum concentration of contrast media to the coronaries.

In 1955 Boerma and Blickman⁵ published their experimental animal work concerned with elevation of the intrabronchial pressure and the consequential reduction in intrathoracic circulation as an aid in angiocardiology. In 1960 Nordenström⁶ published his more extensive monograph on elevated intrabronchial pressure during contrast examina-

tion of the cardiovascular system, and in that publication he includes a section on coronary angiography in man, utilizing the reduction of systemic flow and the subsequent layering of the relatively heavy contrast medium. When the coronary arteries are dependent to the point of contrast injection, that is, when the patient is upright or in the prone oblique position, and contrast deposition is in the supravulvar thoracic aorta, the contrast will flow with the aid of gravity into even rather severely diseased arteries. As described by Nordenström, Ovenfors, and Törnell⁷ in their experience with this procedure in 100 cases of ischemic heart disease, the method is as follows:

By Seldinger's⁸ method of percutaneous catheter placement, a radiopaque catheter is inserted into the femoral artery and guided under fluoroscopic control to a position just above the aortic valves; the catheter has an end opening with several side holes near the tip. A second catheter, this one of thin polyethylene, is also inserted into the femoral artery just distal to the original catheter used for contrast injection; this catheter is for continuous recording of the systemic blood pressure. A test dose of contrast material is injected by hand to insure proper position of the radiopaque catheter. If the patient is able to execute a satisfactory Valsalva maneuver so that the blood pressure falls to 80-100 mm. Hg, the examination may be performed with the subject sitting and the

From the Division of Radiology and Nuclear Medicine

¹ Assistant Attending Radiologist, Presbyterian-St. Luke's Hospital
Clinical Instructor, Department of Radiology, University of Illinois College of Medicine

left anterior portion of the chest against the film changer. In other patients, general anesthesia is induced with intravenous injection of sodium pentobarbital, followed by a muscle relaxant, and the patient then intubated with a cuffed endotracheal tube. The tube is connected to a specially constructed, oxygen injecting device for controlled inflation of the lungs and elevation of the intrabronchial pressure, and the patient is positioned prone oblique with the left anterior chest resting upon the horizontal filming plane. The EKG, blood pressure, and intrabronchial pressure are observed on the oscilloscope, as well as permanently recorded. The intrabronchial pressure is elevated to 40-50 cm. of water, and the blood pressure usually promptly falls to about 70 mm. Hg. At this point, 1 ml. per kilogram of body weight of a 76% solution of Urografin (Schering) is in-

jected with the pressure syringe within about three seconds. The blood pressure is generally not reduced for more than approximately ten seconds and returns promptly to a normal level after the intrabronchial pressure is reduced. The filming is accomplished with a rapid changing device and specially mounted, stereoscopically positioned tubes. No serious complications were observed during this series of patients.

Figure 1 is a recording strip from a coronary arteriogram performed on a dog during the above described conditions; these events are quite similar to those observed in the human. Note the reduction in heart rate and diminution of the R wave on the electrocardiogram, as well as the prompt response of the blood pressure to the intrabronchial pressure variations.

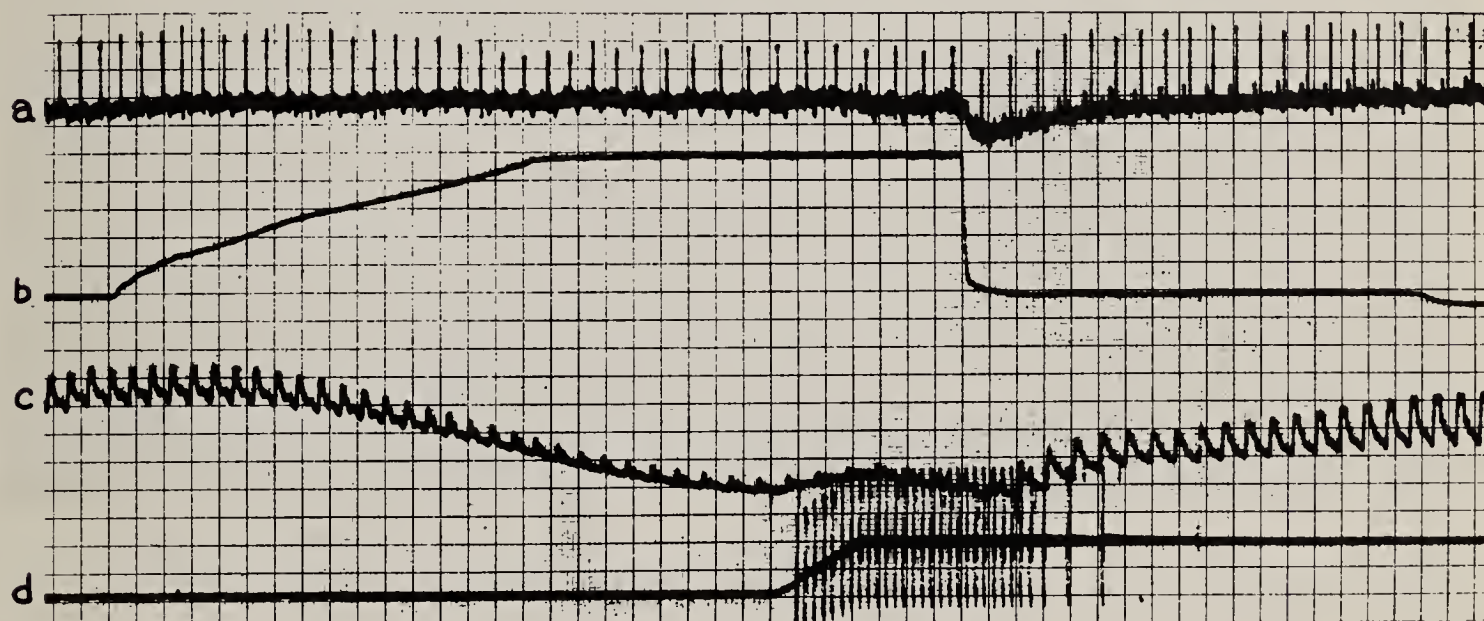


Fig. 1.—Graphic recording of a coronary arteriogram. a. Lead 2 EKG. b. Intrabronchial pressure. Base line—0; plateau—50 cm. H₂O. c. Systemic blood pressure. d. Injection and exposures. The upward deflection is the injection time; the multiple vertical lines are the exposures.

Paper speed—1 cm. (2 large squares) per second.

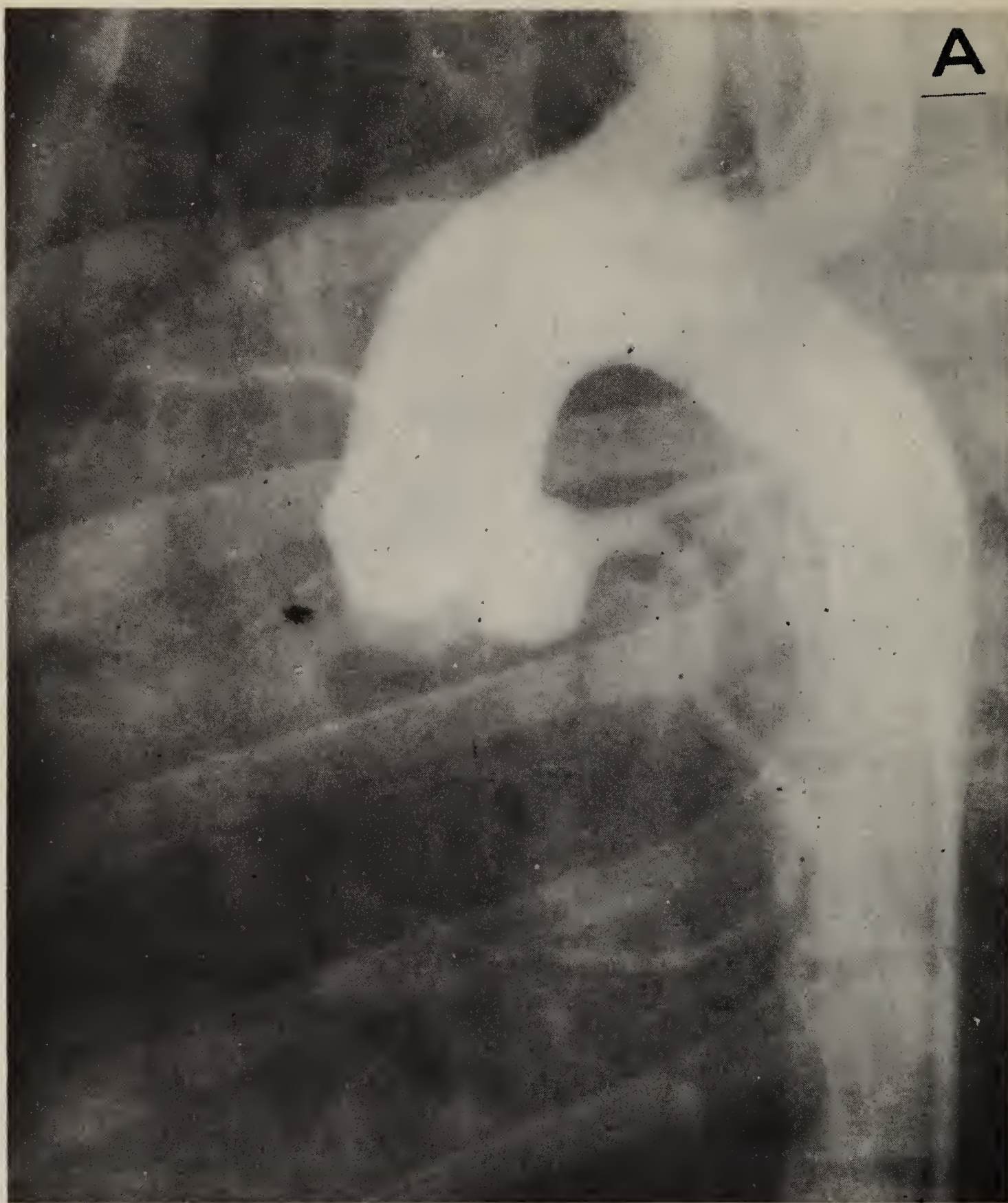


Fig. 2.—Examples of coronary arteriograms illustrating the effects of elevation of the intrabronchial pressure. A—without intrabronchial pressure elevation.

Figure 2 illustrates the effects of intrabronchial pressure elevation during coronary arteriography. The representative films are from the same dog and at exactly the same time in the filming sequence of two successive arteriograms; all factors are as identical as possible, with the exception of the variation in intrabronchial pressure. Note the mark-

edly improved coronary filling in B, as well as the improvement of the general radiographic appearance. Note also the diminution in caliber of the intrathoracic vessels, in addition to overall reduction in comparative heart volume. There is a slight distortion of the coronary vessels during the “squeezing” effect of the inflated lungs, but this is not considered

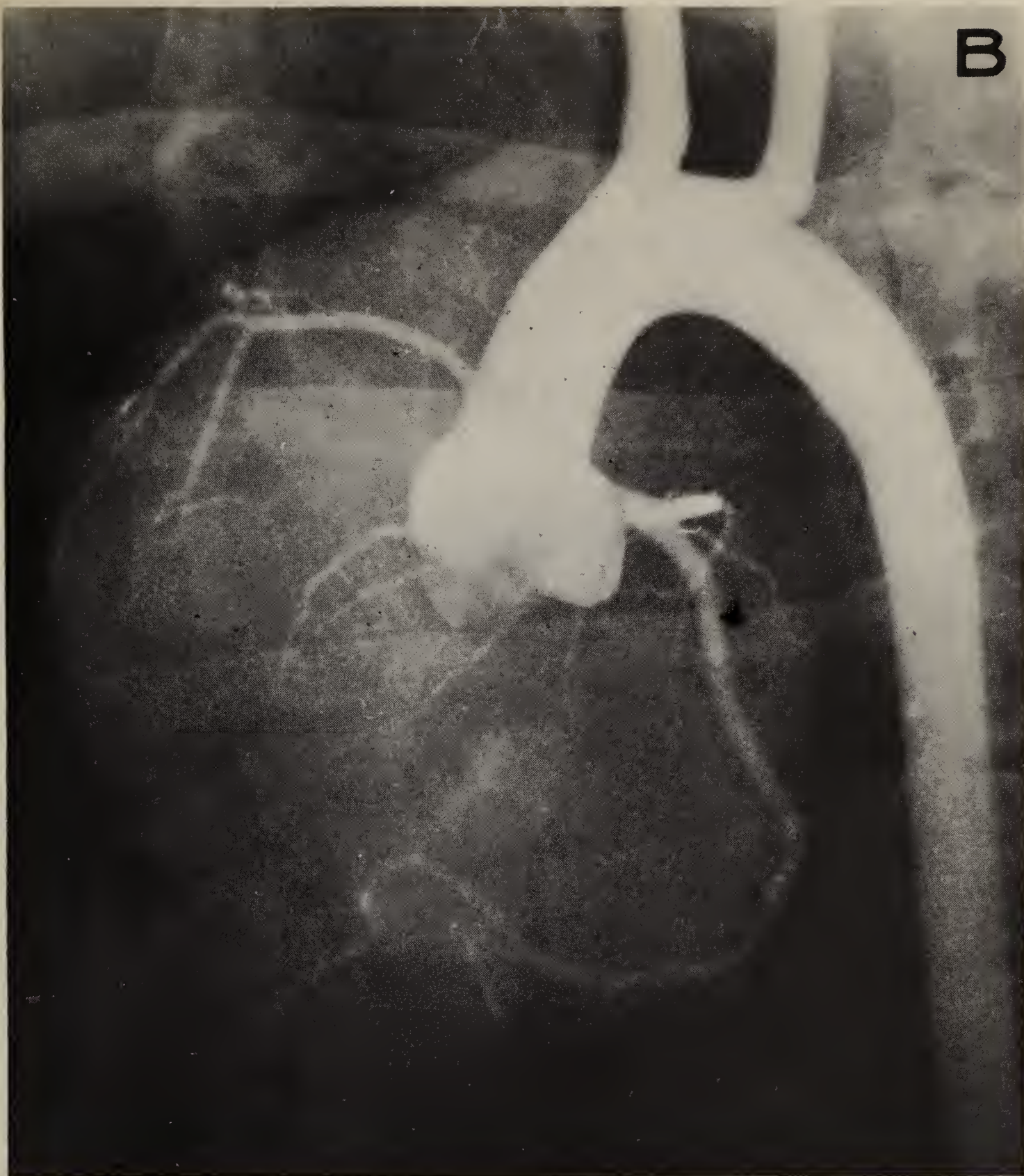


Fig. 2—Examples of coronary arteriograms illustrating the effects of elevation of the intrabronchial pressure. B—with intrabronchial pressure elevation.

to adversely affect the clinical interpretation of the material. The predominance of the left coronary system in the dog as noted on these films is observed only in about five per-cent of humans, predominantly males, according to James.⁹

It is not the purpose of this introductory paper to discuss the indications, clinical value, and future of this examina-

tion; it is apparent, however, that this procedure provides more than simple visualization of the anatomic status of the proximal, main stem coronaries. Figure 3, for example, is a portion of a human arteriogram clearly illustrating the artery to the A-V node, which arises from the "artery of the U," in this case a division of the right coronary artery. The

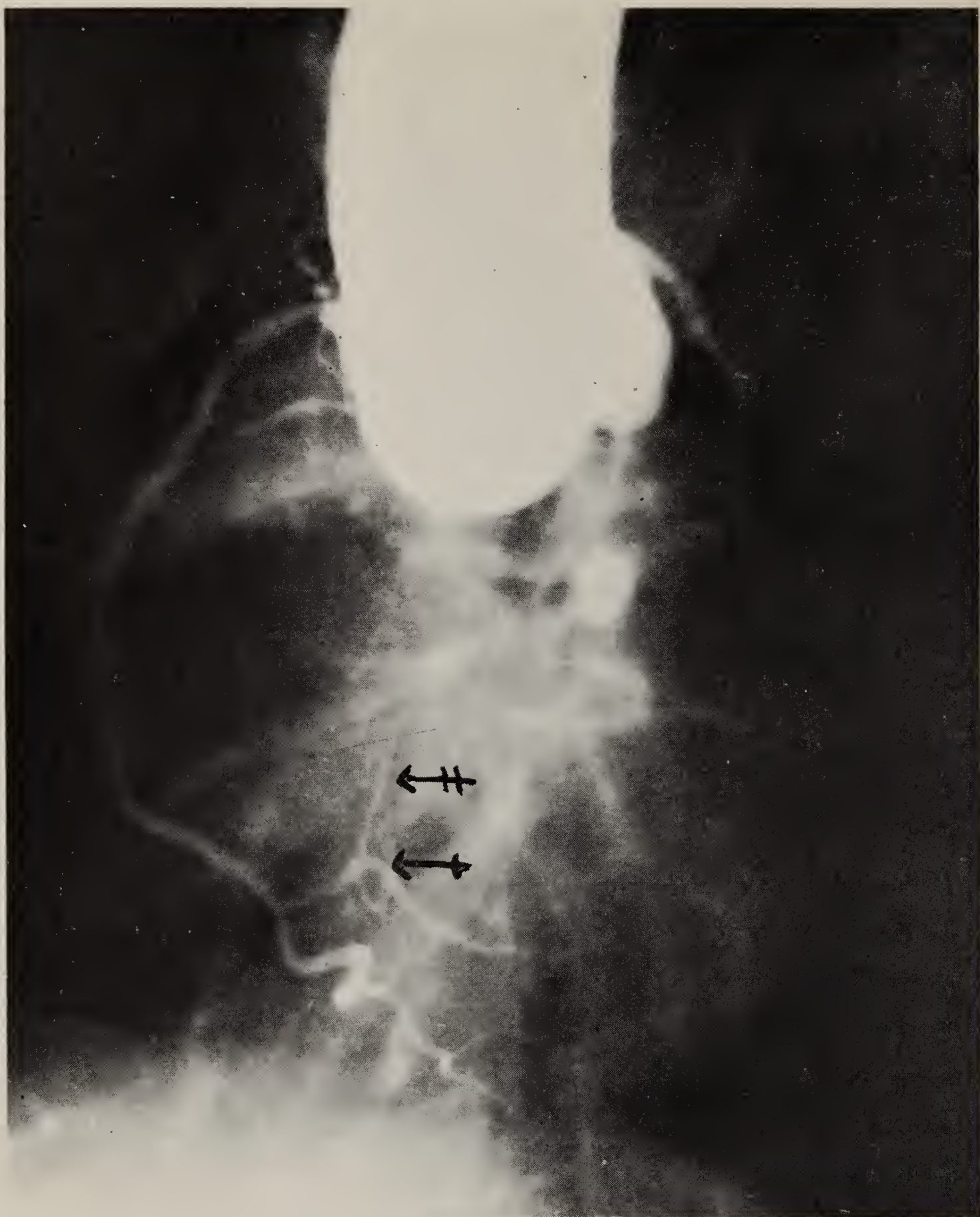


Fig. 3.—Human coronary arteriogram demonstrating the artery of the "U" (←+) and artery to the A-V node (←++).

consistent appearance of this artery at the crux of the heart can be a useful landmark in interpreting angiocardio-grams¹⁰—a line from the apex of the U-turn to the apex of the non-coronary sinus divides the right from the left A-V orifice and also generally divides the interatrial from the interventricular orifice.

The cardiac veins are also frequently visualized during the course of the arteriograms above described, but these structures are not as yet of specific diagnostic value.

Nordenström et al¹¹ noticed an accumulation of contrast material in the myocardium later in the filming series in 15 of the previously mentioned 100

cases of coronary arteriograms. They have termed this the "myocardiographic phase" and have postulated it may be due to opacification of the myocardial capillaries (Fig. 4). Because of the implied value of the myocardiogram in diagnosis of involvement of the heart muscle itself, an experiment was designed to investigate the improvement of the appearance of this phase of the coronary arteriogram. It had been initially observed that the degree of opacification of the ventricular muscle was most pronounced immediately after the sudden deflation of the lungs and resultant reduction of the elevated intrabronchial pressure. Since the inflated lungs do exert a compressing effect on the heart, it was reasoned that the abrupt expan-

sion of the vascular bed of the myocardium occurring just following the prompt return of the inflated lungs to normal, might be actively responsible for "sucking" the contrast material into the smaller vessels of the muscle mass, similar to the prompt fill of a sponge which has been squeezed and then rapidly allowed to expand in a fluid environment. It was further postulated that if the blood flow to the heart were diminished prior to the sudden deflation of the lungs, contrast material which had been deposited in the supravulvular aorta would be present in even greater percentage for presentation to the smaller vessels. With this basis, the experiment was conducted as follows:



Fig. 4.—"Myocardiogram" of the left ventricular wall. (Reproduced with permission of Nordenström)

Coronary arteriography was performed on eight mongrel dogs under preliminary circumstances as outlined in the text. In one half the group, the vena cavae were blocked with balloon catheters distended with contrast material and positioned under fluoroscopic control, followed by inflation of the lungs to a pressure of 50 cm. H₂O, then contrast was injected in the aortic root, and filming commenced, as illustrated in Figure 5. Midway in the filming program, the insufflated lungs were quickly deflated by disconnecting the airway tube from the oxygen injector. In the other group, the lungs were initially inflated, then the vena cavae blocked, followed by contrast

injection, deflation of the lungs, and filming. In both groups the great vein occlusion was continued throughout the filming period.

Figure 6 (A and B) are representative recording strips from each of the two experimental groups. An early observed, bothersome complication was fatal ventricular fibrillation, as noted in Figure 6B; this event was noted in a part of each of the two groups of animals, and was assumed due to the resultant hypoxia of the experimental conditions. In addition to this complication, no improvement of the myocardiogram was noted in either group, and the experiment was abandoned.

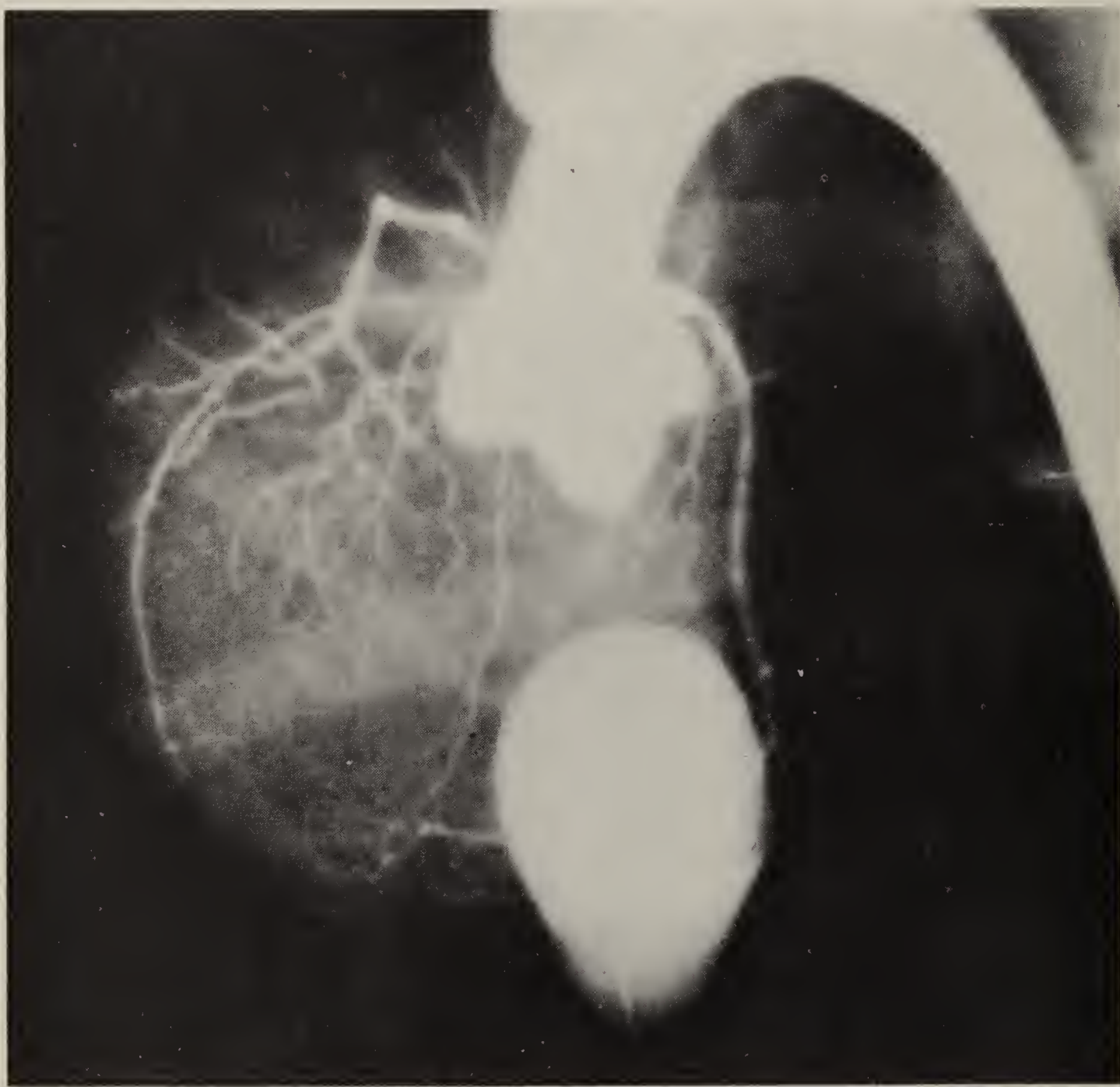


Fig. 5.—Coronary arteriogram in the dog with opacified balloon catheters occluding the superior and inferior vena cavae, and also with the lungs inflated.

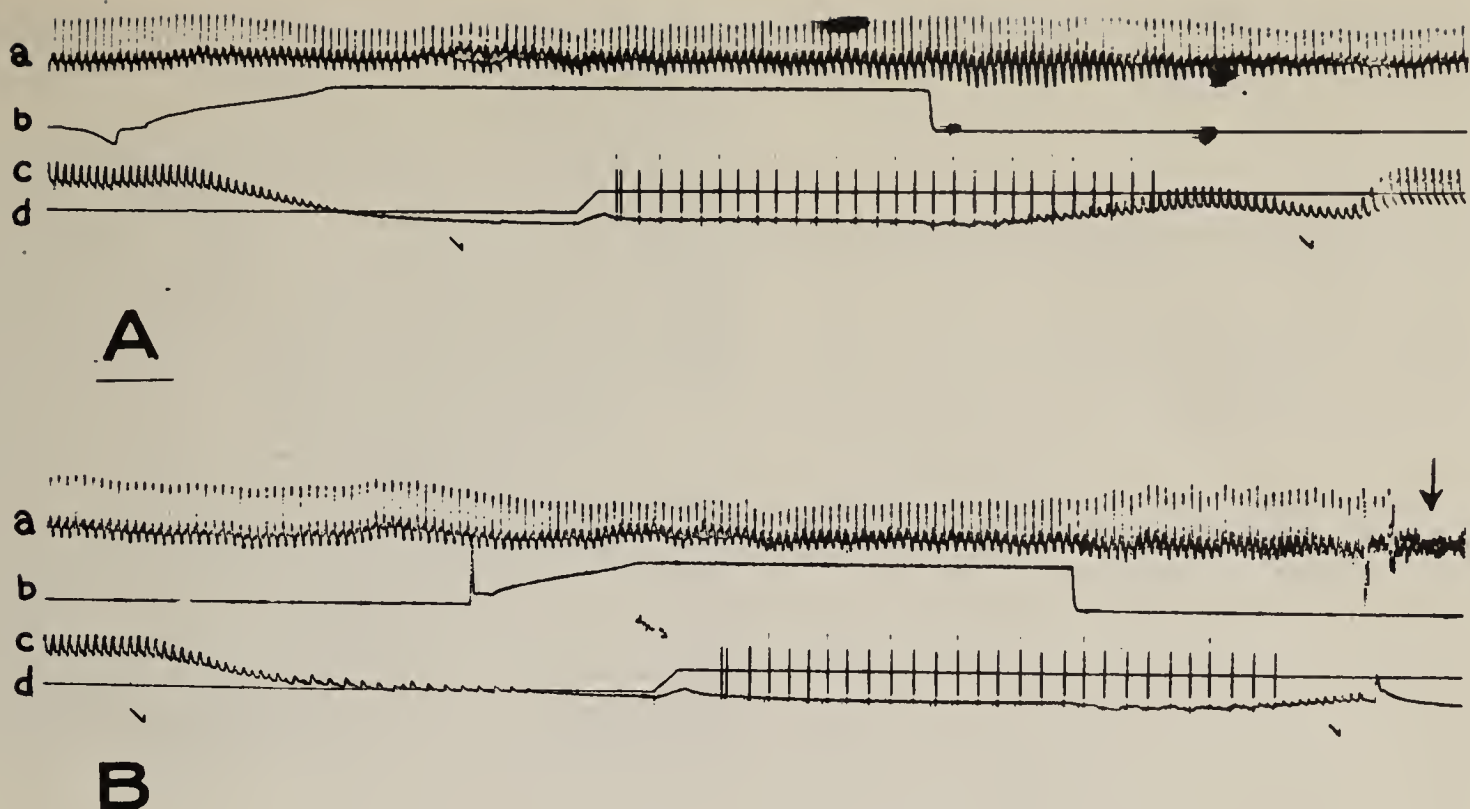


Fig. 6.—Recordings of two arteriograms utilizing block of the vena cavae and elevation of the intrabronchial pressure. a. Lead 2 EKG. b. Intrabronchial pressure. Base line—0; plateau—50 cm. H₂O. c. Systemic blood pressure. d. Injection and exposures. The upward deflection is the injection time; the multiple vertical lines are the exposures. Paper speed—1 cm. (2 large squares) per second. A. Vena cavae block followed by elevation of intrabronchial pressure. B. Elevation of intrabronchial pressure followed by vena cavae block. Note terminal fatal ventricular fibrillation. (Arrow).

ACKNOWLEDGMENT

The author is grateful to Docent Nordenström and his staff for the opportunity of spending the academic year, 1962-1963 at the Thoracic Clinic in Stockholm, and to the Pillsbury Resident Fund for making that opportunity financially possible.

REFERENCES

1. Roushöi, P.: *Acta Radiologica*, 14:419, 1933.
2. Reboul, H., and Rocine, M.: *Presse Méd.*, 1:763, 1933.
3. Radner, S.: An Attempt at the Roentgenologic Visualization of Coronary Blood Vessels in Man. *Acta Radiologica*, 26:497, 1945.
4. Gensini, G.: Coronary Angiography. *Progress in Cardiovascular Disease* Vol. 6, No. 2:155, 1963.
5. Boerma, I. and Blickman, J. R.: Reduced Intra-thoracic Circulation as an Aid in Angiocardiography. *J. Thorac. Surg.*, 30:129, 1955.
6. Nordenström, B.: Contrast Examination of the Cardiovascular System During Increased Intrabronchial Pressure. *Acta Radiologica*, Supp., 200, 1960.
7. Nordenström, B., Ovenfors, C.-O., and Törnelli, G.: Coronary Angiography in 100 Cases of Ischemic Heart Disease. *Radiology* 78, No. 5:714-724, 1962.
8. Seldinger, S. I.: Catheter Replacement of the Needle in Percutaneous Arteriography. *Acta Radiologica*, 39:368, 1953.
9. James, T. N.: *ANATOMY OF THE CORONARY ARTERIES*. Paul B. Hoeber, Inc. 1961.
10. James T. N.: A Useful Landmark for Interpreting Angiocardiograms. *Radiology*, 79:804, 1962.
11. Nordenström, B., Ovenfors, C.-O., and Törnelli, G.: Myocardiography and Demonstration of the Cardiac Veins in Coronary Angiography. *Acta Radiologica*, 57:11, 1963.

REVIEW ARTICLE

CYTOMEGALIC INCLUSION DISEASE

DONALD HENSON, M.D.¹

Perhaps one of the most common disorders in a premature infant with hepatosplenomegaly, jaundice, and hemorrhagic diathesis is cytomegalic inclusion disease or generalized cytomegalovirus* infection. Although once considered a rare entity in infants, this disease is now recognized to rival both erythroblastosis and rubella in contributing to infant mortality and morbidity. Pathologically, cytomegalic inclusion disease has been well studied; but, clinically and virologically, it is not completely defined and many concepts still highly speculative and associative. In this paper, recent studies of the disease are reviewed, several cases presented, and an attempt made to correlate experimental and clinical findings.

Cytomegalic inclusion disease is a generalized necrotizing viral infection characterized by the formation of large epithelial cells bearing intranuclear and less frequently cytoplasmic inclusions (Fig. 1).¹ In the past, diagnosis was made only at autopsy; but, by isolation of the virus and the use of exfoliative cytology, a positive diagnosis can now be made during life. Although premature infants are most often affected, the disease has also been reported in children and adults. In young infants, the disease is usually the result of an *in utero* infection caused by an inapparent infection in the mother. It can occur as an acute fulminating process which may be rapidly fatal or as a chronic mild infection resulting in permanent central nervous system damage. In some instances recovery may be

complete. In children and adults the disease is often preceded by a severe debilitating disorder such as pertussis or leukemia.

It should be emphasized that the presence of inclusions within the salivary glands of normal children does not indicate cytomegalic inclusion disease (Fig. 2). Indeed, such inclusions are found in the salivary glands of 10% of all infants regardless of the cause of death in some localities.² These inclusions are found in children most frequently between two months and two years of age, and have not been observed after 16 years of age.³ Inasmuch as the inclusions are rarely seen before two months of age, a post-natal infection is assumed to be the cause.⁴

INCIDENCE

Cytomegalic inclusion disease accounts for 1–2% of all deaths in infants as based on autopsy studies.⁵ However, because of the existence of non-fatal cases, the exact incidence of the disease is probably much higher.

From the Division of Pathology

¹ Intern in Pathology, Presbyterian-St. Luke's Hospital

*Originally called salivary gland virus.

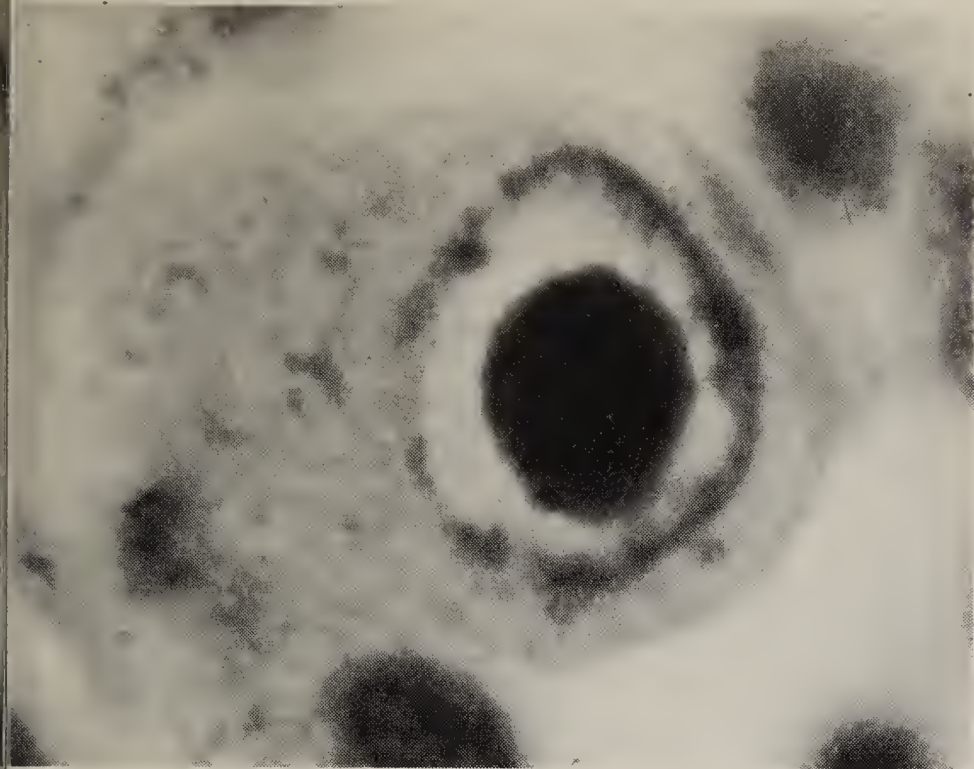


Fig. 1.—Photomicrograph of an alveolar cell from the lung containing a typical intranuclear inclusion. The cell is greatly enlarged, and, within the nucleus, there is a large homogeneous inclusion separated from the nuclear membrane by a clear space or "halo."

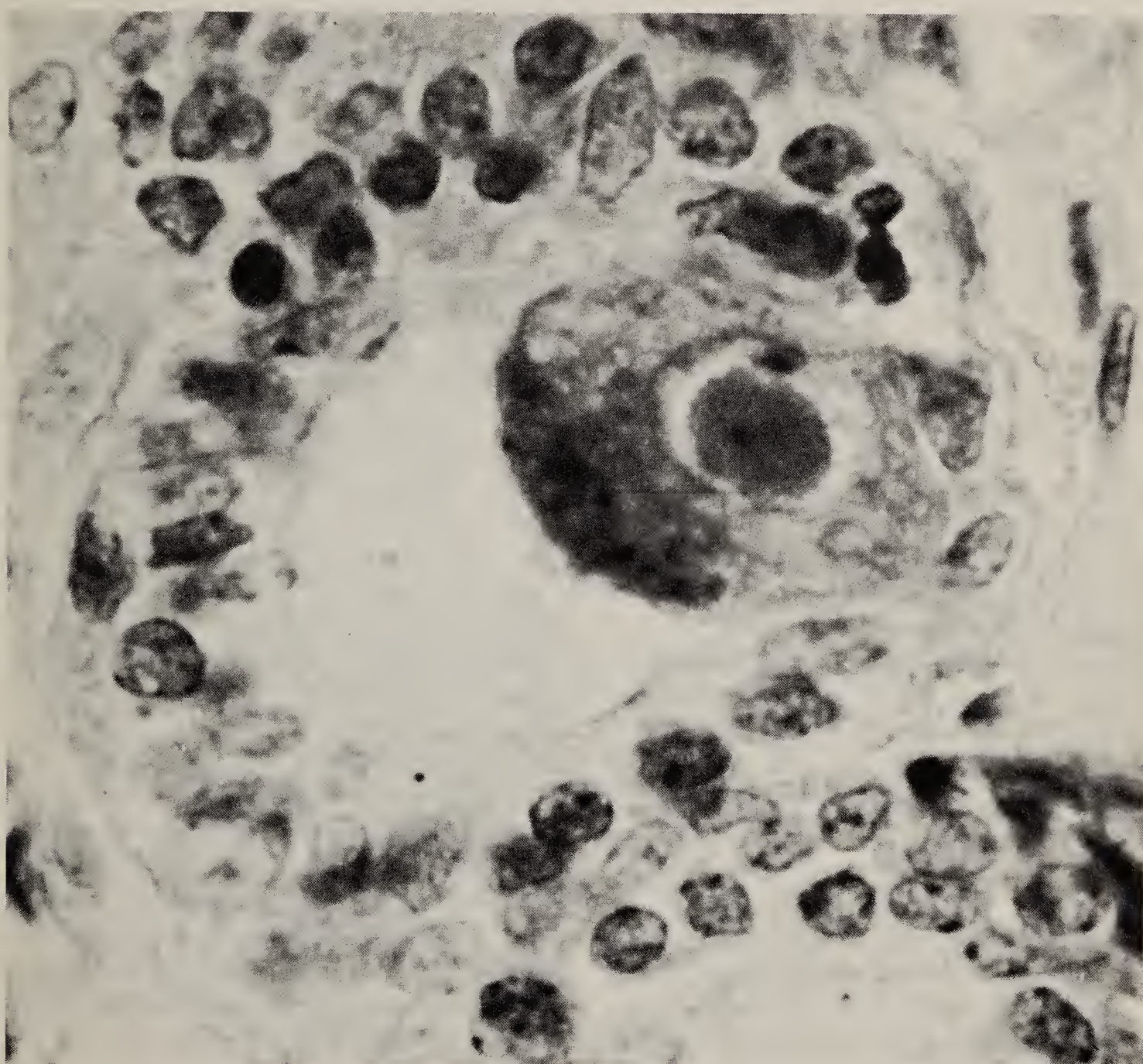


Fig. 2.—Intranuclear inclusion in the ductal epithelium of the parotid gland. Cytoplasmic inclusions are seen near the cell surface as dark staining granular material.

ETIOLOGY

The etiological agent is the human cytomegalovirus which belongs to a group of species specific viruses ubiquitous in distribution.^{1,4,6,7} Other cytomegaloviruses in this group include a murine⁸ and a cavian⁹ strain as well as a virus recently isolated from an African Cercopithecus monkey.¹⁰ The human strain has been well characterized. It is an ether-sensitive¹¹ DNA virus¹² measuring 100 to 125 millimicrons in diameter.¹³ The virus readily grows in tissue cultures of human fibroblasts derived from either adult myometrium or embryonic skin and muscle.^{6,11} Efforts to adopt the human strain to laboratory animals or cultivate the virus in non-human cells have failed.

The etiological relationship to the disease appears well established. The virus has been isolated from patients with morphological evidence of the disease, in tissue culture it produces both cytoplasmic and nuclear inclusions similar to those found *in vivo*, and patients with the disease possess antibodies against established laboratory strains.

CLINICAL SIGNS

The clinical signs have been reviewed by Medearis¹⁴ using 132 autopsy cases and by Weller and Hanshaw⁴ who followed 17 cases that survived.

In young infants, the signs classically associated with the disease are hepatosplenomegaly, petechiae, jaundice and microcephaly. Other findings have included hemorrhages, chorioretinitis, progressive, respiratory distress, cerebral calcification, and hydrocephaly.

Massive hepatosplenomegaly is not uncommon. Petechiae, which are often visible at birth and of variable distribution, are most pronounced during the neo-natal period. Jaundice can appear anytime. It usually lasts several days and occasionally persists for months. Microcephaly, when apparent at birth, usually indicates permanent central nervous system damage in patients who survive. Hemorrhages which often occur termin-

ally are manifested by hematuria, hemoptysis, melena and bleeding from the cord stump. In general, the clinical signs will appear sooner and be more severe in infants under 2500 gms.

In older infants and children, the disease presents with a different clinical pattern.³ This includes hepatosplenomegaly, progressive respiratory difficulty not responding to antibiotics, protracted diarrhea and vomiting, and hepatic insufficiency. Generally, as the age increases, the clinical pattern shifts with progressive pneumonitis or diarrhea becoming the presenting signs (cases 2 and 4). In contrast to newborn infants, the central nervous system is spared and prematurity is not a factor. Not infrequently, the signs will follow a severe systemic disorder with pertussis and fibrocystic disease the two most common reported antecedent diseases.

The prognosis is variable. The infant may die 24 hours after birth, or follow a slow unfavorable course characterized by progressive renal, hepatic, or pulmonary failure. Indications are that a high percentage of patients who recover from congenital infection will have permanent central nervous damage.⁴ Sequelae have included mental retardation, blindness, epileptiform seizures, and spastic paralysis. Indeed, evidence now supports Potter's contention that many institutionalized children, suspected of having the sequelae of erythroblastosis, are survivors of congenital cytomegalovirus infection¹⁵. Premature infants symptomatic at birth have the worst mortality and morbidity.

LABORATORY DATA

Laboratory data usually reveals a mild hypochromic anemia and thrombocytopenia with a leuco-erythroblastosis on peripheral smear. Low plasma proteins and elevated serum bilirubin, particularly the direct fraction, indicate hepatic involvement. Radiographically there may be cerebral calcification and/or hydrocephalus. Tests for Rh and ABO blood incompatibility, including direct and indirect Coombs, are negative.

ADULT CASES

The disease rarely occurs in adults. To date, 54 cases have been reported in the world literature.¹⁶ Clinically, the disease is usually a terminal association with a preceding chronic disorder. Although its clinical features are poorly defined, suspicion should be aroused when a patient develops progressive pneumonitis, enteritis, or hepatitis, superimposed on a debilitating disease. Among the predisposing conditions most frequently cited are leukemias, lymphomas, refractory anemias, and chronic gastrointestinal disorders (case 5).

MORBID ANATOMY

Histologically, the pathognomonic feature is the presence of large eosinophilic intranuclear inclusions occurring within greatly enlarged cells of many organs (Fig. 1).^{1,5} Such inclusions which tend to conform to the shape of the nucleus may be granular or homogeneous and are usually separated from the inside of the nuclear membrane by a clear space or "halo." Because of their large size, these inclusions were once considered protozoa and tentatively classified as *Entamoeba mortinatalium*.¹⁷ Not infrequently, the intranuclear inclusions are associated with numerous small cytoplasmic inclusions within the same cell (Fig. 2).

In stillborn infants and infants dying from congenital infection, nuclear inclusions are found with decreasing order of frequency in the kidneys, liver, lungs, pancreas, thyroid and brain. Rarely, they are seen in the ovaries, intestine, pituitary, thymus, skin, heart and parathyroids. In general, organs having the richest blood supply are most frequently affected.

The most common pathological findings are an interstitial nephritis, interstitial pneumonitis (Fig. 3), hepatic cell necrosis, and cerebral calcification. Extensive *in utero* involvement often leads to gross malformations of the central nervous system. Micropolygyria, hypoplasia, and hydrocephalus are most frequent. An ependymitis is probably the

basis for the hydrocephalus.

In contrast to infants, the organs most frequently affected in children and adults are lungs, adrenals, liver, alimentary tract, spleen, pancreas and kidneys. Alveolar proteinosis and hepatoadrenal necrosis are the two most frequent post-mortem diagnoses.³

CLINICAL VIROLOGY

Preliminary evidence indicates that cytomegalovirus is widely disseminated throughout the population. The frequency of complement-fixing antibodies in various age groups is shown in Fig. 4. In addition, Rowe *et al* recovered virus from the saliva or urine of 28% of healthy institutionalized children 8 months to 4 years of age.¹⁸ In fact, many children possessed complement-fixing antibodies at the time of virus isolation, and some excreted virus continually for 5 months even with significant levels of antibodies.

Cytomegalovirus has also been isolated in tissue cultures of human adenoids excised from apparently healthy children. On this basis, it has been suggested that cytomegalovirus may persist in lymphoid tissue.¹¹

Hanshaw and Weller have obtained virus from the urine of 4 out of 50 leukemic children, none of whom possessed antibodies.¹⁹ In one case, virus isolations extended over a four month period; and, in another, isolations coincided with an exacerbation of the leukemic process. Interestingly, in two of three patients followed to autopsy, no morphological evidence of the disease could be found.

Chronic infections have also been demonstrated in animals. Brodsky and Rowe consistently obtained virus from the salivary glands and occasionally from the urine and saliva of mice for one year following inoculation with the murine cytomegalovirus.²⁰

From these preliminary studies, it is apparent that cytomegalovirus enjoys an unique host-parasite relationship. Not only is there a tendency for the host to excrete virus for long periods despite high levels of circulating antibody, but many cytomegalovirus infections are chronic,

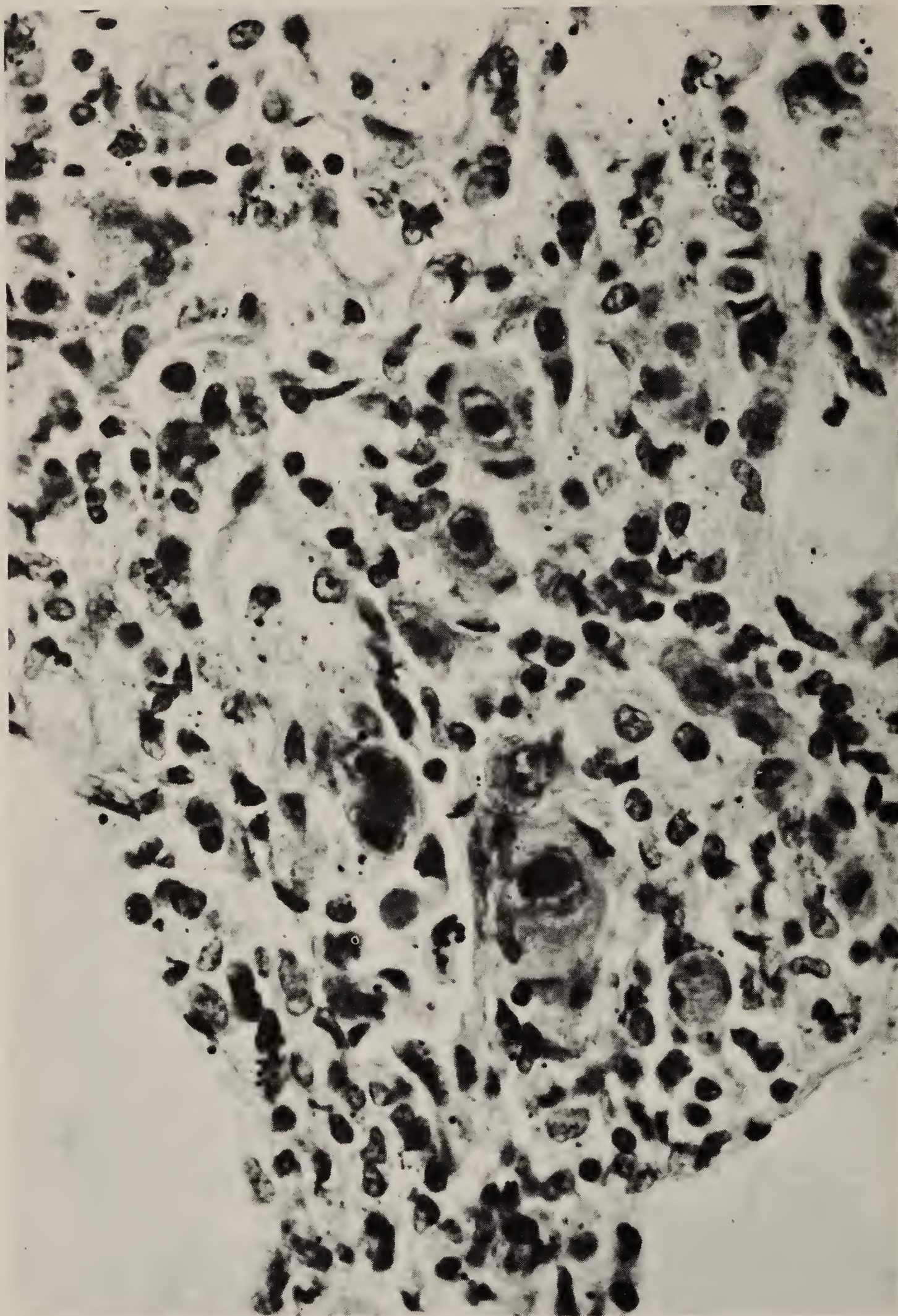


Fig. 3.—Interstitial pneumonitis from case 2. Note several large inclusion-bearing cells surrounded by a mononuclear cell infiltrate.

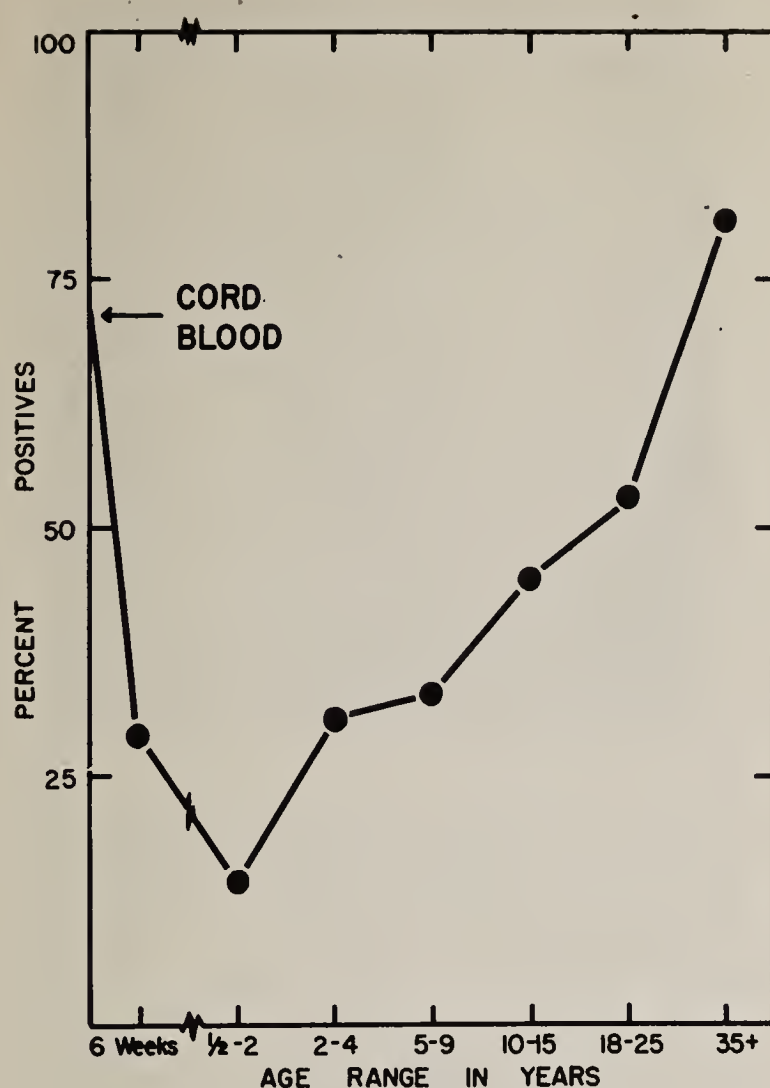


Fig. 4.—Percent of individuals in various age groups with complement-fixing antibodies against one strain of cytomegalovirus. Data from Rowe *et al.* The abscissa is not drawn to scale.

asymptomatic and generalized. Of primary importance and yet to be defined are those factors which determine whether the infection remains chronic and asymptomatic, or occurs as a generalized necrotizing disease. Some of the factors thought responsible are briefly reviewed in the next section.

PATHOGENESIS

From the fact that many cases have been preceded by a debilitating disorder, particularly of the reticuloendothelial system, Nelson and Wyatt postulated that disseminated disease in adults is caused by an immunological deprivation resulting in reactivation of a latent infection.³ The theory is attractive, especially since reticuloendothelial diseases are frequently associated with other latent infections, viz., herpes zoster, cryp-

tococcosis. In addition, many patients had received steroids, and other chemotherapeutic agents capable of depressing the immunological system.¹⁶ The adverse effect of steroids on viral diseases has been well documented.²¹

The theory proposed by Nelson and Wyatt is now being approached experimentally using the murine strain of cytomegalovirus as an experimental tool. The effect of injecting aminopterin into mice during the acute phase of the disease is shown in Fig. 5. The effect of aminopterin during the chronic phase is yet to be defined experimentally.

Another theory relating the frequent association of cytomegalic inclusion disease with debilitating diseases has been advanced by Pearce.²² He suggested that infection may result when a debilitated patient receives blood from an unsuspected cytomegalovirus carrier.

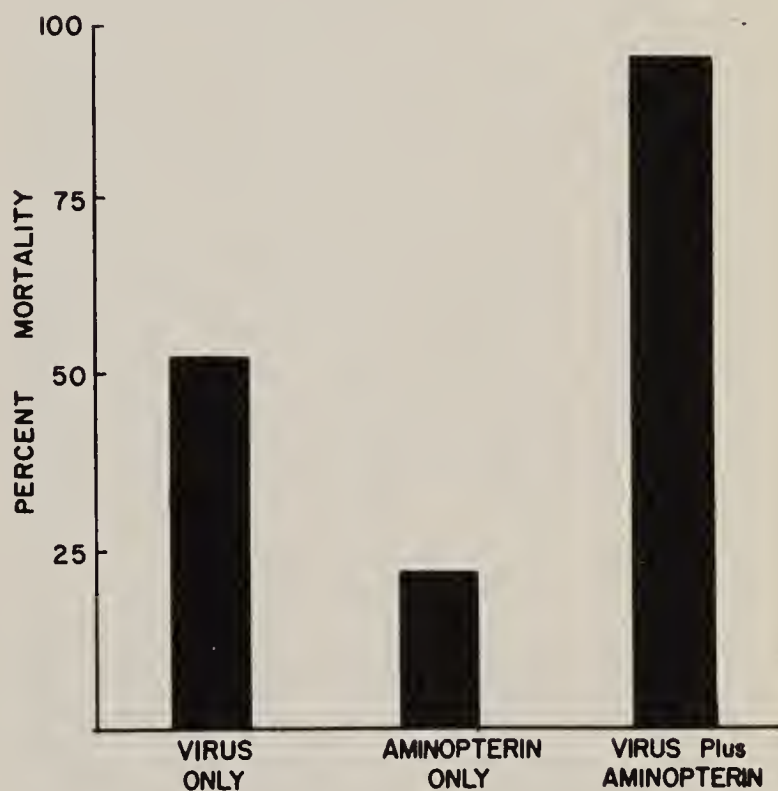


Fig. 5.—Result of injecting aminopterin, an inhibitor of the reticuloendothelial system, into mice 2 days after receiving a subcutaneous injection of murine cytomegalovirus. Observe that the mortality rate 6 days after infection is greater in this group than the combined mortality of those mice which received either virus or aminopterin only.

The pathogenesis of viral dissemination in pregnant women with transmission across the placenta is purely speculative. It is interesting to note that of approximately 60 cases in newborn infants that have been described, six have occurred in three sets of twins; a frequency higher than the ratio of twin to single births.²³ Seifert and Oehme point out that the frequency of twinning in Germany is 1 in 81 births while the frequency of cytomegalic inclusion disease in twins is 1 in 9.5 cases.

Evidence is now available to indicate transplacental passage. Kramer has encountered murine cytomegalovirus in tissue cultures prepared from mouse embryos,²⁴ and the author has seen a case in a newborn in which inclusions were found in the placenta.²⁴ This latter observation may indicate that cytomegalovirus reaches the fetus by first replicating within the placenta, and not by free transmission.

It is not clear why the incidence is higher in premature infants or what role prematurity plays in the disease. Experimentally, injection of murine cytomegalovirus into newborn mice resulted in a marked growth retardation, apparent several days after birth (Fig. 6). Histological examination of the infected mice revealed typical inclusions in almost every organ. Extrapolation of these data to congenital infection in man suggests that prematurity may be the result and not the cause of the disease.

DIAGNOSIS

In infants the disease can be diagnosed by virus isolation, cytological demonstration of typical inclusions, or classical radiographic findings. However, because of widespread dissemination of cytomegalovirus in the population, a positive diagnosis should be made only in the presence of definite clinical and pathological disease patterns.

With tissue culture facilities, cytomegalovirus can be isolated from the urine, saliva, or biopsies of involved organs.^{4,6} *In vitro* the virus is readily identifiable by its unique cytopathology; and, there-



Fig. 6.—Effect of injecting the murine cytomegalovirus into newborn mice. Note the growth retardation in the infected mouse on the left as compared to control. Photograph taken 7 days after infection.

fore, neutralization tests are not necessary.

It is suggested that in a suspected case, 1) repeated attempts at isolation be made particularly from the urine; and 2) most liver biopsies in children be cultured routinely for cytomegalovirus. It must be stressed however, that isolation of cytomegalovirus may reflect an inapparent infection, completely unrelated to the disease under study.

The disease can be diagnosed by finding inclusion-laden cells in stained urinary sediment.²⁵ However, as emphasized by Weller, not all patients have demonstrable cells, even in the presence of persistent viruria.⁴ For optimal results, several specimens of freshly voided urine should be submitted to the pathologist. Characteristic inclusional cells may also occur in spinal fluid, bronchial and gastric washings.³

A presumptive diagnosis can be made radiographically with findings of focal peri-ventricular cerebral calcification. The distribution of cerebral calcification in the subependymal mantle can serve to differentiate cytomegalic inclusion disease from congenital toxoplasmosis.^{26,27}

The use of serological methods are of

questionable value because of transplacental antibodies, the presence of more than one serological virus type,²⁸ and the lack of adequate reference antisera.

Because of its rare occurrence in adults, cases have been diagnosed only by finding inclusions in post-mortem or surgical material.¹⁶ Exfoliative cytological studies on bronchial washings may show inclusions; however, the infrequent occurrence of inclusions in the kidneys makes diagnosis by examining stained urinary sediment highly improbable.

THERAPY

No specific therapy exists for cytomegalic inclusion disease. Admittedly, therapy is difficult because the disease is often diagnosed late in its course, after widespread viral dissemination.

Nelson and Wyatt reported that large doses of Terramycin (oxytetracycline) greatly prolonged life in experimentally infected mice.³ Attempts to confirm these findings have been equivocal. In tissue culture oxytetracycline inhibited viral replication only at concentrations deleterious to the cell.

Clinically it has been both affirmed and denied that corticosteroids benefit the thrombocytopenia, anemia, and hepatitis.^{4,29,30} In general, therapy must be supportive requiring measures to control bacterial infection, kernicterus, dehydration, anemia, and other possible complications. Drugs which tend to depress the reticuloendothelial system should be avoided.

CASE REPORTS

The following cases are presented in abstract form in order to illustrate both the clinical and pathological variations of the disease. Four cases have been taken from the literature. One (case 2) is from the files of Presbyterian-St. Luke's Hospital.

CASE 1¹⁴

A white male infant weighing 2098 gms. was born to an 18 year old mother who received a blood transfusion 7 months prior to delivery. At birth, the infant's

condition was satisfactory in spite of mild cyanosis. However, within hours, the infant became lethargic, jaundiced, edematous, and developed multiple petechiae. The liver and spleen were palpable 2 cm. below the costal margin. The infant expired 3 days later following massive hematemesis. At autopsy, characteristic inclusions were found in the brain, liver, kidneys, lung and thyroid.

CASE 2

A 2 month old Negro male infant (birth weight 3740 gms) was seen in the clinic because of diarrhea with a subsequent diagnosis of gastroenteritis, cause unknown. Ten days later, during which time the diarrhea had gradually subsided, the infant developed sudden respiratory distress with cyanosis. The child expired on admission. Autopsy revealed aspiration pneumonitis plus typical cytomegalic inclusions in the lungs, adrenals, small intestine, and kidneys.

CASE 3³¹

A white male infant (birth weight 3000 gms) was born to an 18 year old primigravida whose pregnancy was complicated by respiratory and urinary tract diseases. Some 6 hours after birth, jaundice and a petechial rash most marked about the head and face were noted. The white count was 14,200, hemoglobin 18 gm%, and the platelet count 124,720/mm³. Urinalysis was negative except for slight proteinuria during the first 4 days. The jaundice clinically disappeared by the 7th day, but the hepatosplenomegaly increased.

Virus was isolated from the urine on day 14, from a liver biopsy on day 25, and again from the urine after approximately 1, 3, 16 and 26 months of age. Neutralizing antibody against the virus was demonstrated in the patient's serum during the time of viruria. No inclusions were seen in stained sections of the liver biopsy. At 26 months of age there was impairment of speech development, a head circumference below the third percentile and hepatomegaly.

CASE 4¹⁹

A 7 year old boy, who was diagnosed as having acute leukemia 6 months pre-

viously, was hospitalized with fever and respiratory distress. Respirations were shallow and increased in rate. Chest films revealed extensive pulmonary infiltration of both lower lobes. Bacteriological studies were negative. On the 8th and 23rd hospital day, cytomegalovirus was isolated from the urine. The patient died 8 weeks later and, at autopsy, inclusions were found only in the lungs.

CASE 5³²

An 18 year old male had a colectomy for ulcerative colitis. On histological examination characteristic cytomegalovirus inclusions were found in the surgical specimen. Attempts to culture the virus from the urine, frozen surgical specimen and post operative biopsy material failed; and antibodies could not be detected. However, within a year after surgery, antibodies appeared in the patient's serum.

SUMMARY

Cytomegalic inclusion disease can occur at all ages. In young infants, the disease is characterized by hepatosplenomegaly, jaundice, hemorrhage, and cerebral calcification. Survivors may be mentally retarded. In children and adults, the disease which usually follows a severe debilitating disorder has a poorly-defined clinical pattern that includes pneumonitis, hepatitis, and enteritis. Frequently it

occurs as a terminal complication. In infants, cytomegalic inclusion disease is the result of a congenital infection; while in children and adults, it is suspected of being the result of a reactivation of a latent virus. The etiological agent has been isolated and characterized. Indications are that it enjoys a unique host-parasite relationship.



REFERENCES

1. Smith, M. G.: Salivary Gland Viruses of Man and Animals (Cytomegalic inclusion Disease). *Progress in Medical Virology*, Vol. 2, pp. 171-202, 1959.
2. Farber, S., and Wolbach, S. B.: Intranuclear and Cytoplasmic Inclusions ("protozoan-like bodies") in the Salivary Glands and Other Organs of Infants. *Am. J. Path.*, 8:123, 1932.
3. Nelson, J. S., and Wyatt, J. P.: Salivary Gland Virus Disease. *Med.* 38:223, 1959.
4. Weller, T. H. and Hanshaw, J. B.: Virologic and Clinical Observations on Cytomegalic Inclusion Disease. *New Eng. J. of Med.*, 266:1233, 1962.
5. Wyatt, J. P., Saxton, J., Lee, R., and Pinkerton, H.: Generalized Cytomegalic Inclusion Disease. *J. Ped.*, 36:271, 1950.
6. Smith, M. G.: Propagation in Tissue Culture of Cytopathogenic Virus from Human Salivary Gland Virus Disease. *Proc. Soc. Exptl. Biol. and Med.*, 92:424, 1956.
7. Weller, T. H., Macauley, J. C., Craig, J. M., and Wirth, P.: Isolation of Intranuclear Inclusion Producing Agents from Infants with Illness Resembling Cytomegalic Inclusion Disease. *Proc. Soc. Exptl. Biol. and Med.*, 94:4, 1957.
8. Henson, D. and Pinkerton, H.: Characteristics of a Plaque Method for the Murine Salivary Gland Virus. *Proc. Soc. Exptl. Biol. and Med.*, 114:130, 1963.
9. Hartley, J. W., Rowe, W. P., and Huebner, R. J.: Serial Propagation of the Guinea Pig Salivary Gland Virus in Tissue Culture. *Proc. Soc. Exptl. Biol. and Med.*, 92:281, 1957.
10. Black, P. H., Hartley, J. W., and Rowe, W.: Isolation of a Cytomegalovirus from African Green Monkey. *Proc. Soc. Exptl. Biol. and Med.*, 112:601, 1963.
11. Rowe, W. P., Hartley, J. W., Waterman, S., Turner, H. C. and Huebner, R. J.: Cytopathogenic Agent Resembling Human Salivary Gland Virus Recovered from Tissue Cultures of Human Adenoids. *Proc. Soc. Exptl. Biol. and Med.*, 92:418, 1956.
12. Goodheart, G. C., Filbert, J., and McAllister, R. M.: Human Cytomegalovirus. Effects of 5-Fluorodeoxyuridine on Viral Synthesis and Cytopathology. *Virology*, 21:530, 1963.
13. Smith, K., and Rasmussen, L.: Morphology of Cytomegalovirus (Salivary Gland Virus). *J. Bact.*, 85:1319, 1963.
14. Medearis, D. N.: Cytomegalic Inclusion Disease: Analysis of Clinical Features. Based on Literature and Six Additional Cases. *Ped.*, 19:467, 1957.
15. Potter, E. L.: Placental Transmission of Viruses: With Special Reference to Intrauterine Origin of Cytomegalic Inclusion Body Disease. *Am. J. Obst. and Gynec.*, 74:505, 1957.
16. Wong, T. and Warner, N.: Cytomegalic Inclusion Disease in Adults. *Arch. Path.*, 74:403, 1962.
17. Smith, A. J. and Weidman, F. D.: Infection of a Stillborn Infant by an Amebiform Protozoan. *Univ. Penn. Med. Bull.*, 23-285, 1910.
18. Rowe, W. P., Hartley, J. W., Cramblett, H. G. and Mastrotta, F. M.: Detection of Human Salivary Gland Virus in the Mouth and Urine of Children. *Am. J. of Hygiene*, 67:57, 1958.
19. Hanshaw, J. B. and Weller, T. H.: Urinary Excretion of Cytomegalo-Viruses by Children with Generalized Neoplastic Disease: Correlation with Clinical and Histopathologic Observations. *J. Ped.*, 58:305, 1961.
20. Brodsky, I., and Rowe, W. P.: Chronic Sub-clinical Infection with Mouse Salivary Gland Virus. *Proc. Soc. Exptl. Biol. and Med.*, 99:654, 1958.
21. Kass, E. H. and Finland, M.: Adrenocortical Hormones in Infection and Immunity. *Ann. Rev. Microbiol.*, 7:361, 1953.
22. Peace, R. J.: Cytomegalic Inclusion Disease in Adults. *Am. J. of Med.*, 24:48, 1958.
23. McAllister, R. M., Wright, H. T. and Tasem, W. M.: Cytomegalic Inclusion Disease in New-born Twins. *J. Ped.*, 64:278, 1964.
24. Personal Communication.
25. Fetterman, G. H.: A New Laboratory Aid in the Clinical Diagnosis of Inclusion Disease of Infancy. *Am. J. Clin. Path.*, 22:424, 1952.
26. Daurelle, G., Smith, G. F. and Riemer, W.: Periventricular Calcification and Cytomegalic Inclusion Disease in Newborn Infant. *J. A. M. A.*, 167:989, 1958.
27. Allen, J. H. and Riley, H. D.: Generalized Cytomegalic Inclusion Disease. *Radiology*, 71:257, 1958.
28. Weller, T. H., Hanshaw, J. B. and Scott, D. E.: Serologic Differentiation of Viruses Responsible for Cytomegalic Inclusion Disease. *Virology*, 12:130, 1960.
29. Gelderen, H. H. van: Successfully Treated Case of Cytomegalic Disease in a New Born Infant. *Acta Ped.*, 48:169, 1959.
30. Margileth, A. M.: Diagnosis and Treatment of Generalized Cytomegalic Inclusion Disease of Newborn. *Pediatrics*, 15:270, 1955.
31. Kluge, R. C., Wicksman, R. S. and Weller, T. H.: Cytomegalic Inclusion Disease of Newborn: Report of Case with Persistent Viruria. *Pediatrics*, 25:35, 1960.
32. Powell, R., Warner, N., Levine, R., and Kirsner, J.: Cytomegalic Inclusion Disease and Ulcerative Colitis: Report of a Case in a Young Adult. *Am. J. Med.*, 30:334, 1961.
33. Seifert, G. and Oehme, J.: *PATHOLOGIE UND KLINIK DER CYTOMEGALIE*. Veb Georg Thieme-Leipzig, 1957.

ABSTRACTS AND REVIEWS

OF PUBLICATIONS BY THE STAFF

The Relationship of "Chronic Pyelonephritis" to Chronic Potassium Deficiency. R. C. MUEHRCKE and J. C. McMILLAN, *Ann. Intern. Med.* 59, No. 4, pp. 427-448, October, 1963.

A review of the literature was made of the multiple functional and structural dearrangement of the kidney of acute potassium deficiency in man and animals. Next, thirty-five patients with prolonged potassium depletion reported in the literature were reviewed. Hydropic vacuolar degeneration was found in the kidneys of twenty out of thirty-five potassium deficient patients. In a large portion of these patients either pyelonephritis or interstitial fibrosis was found.

Serial clinicopathological correlative studies were made on renal tissue obtained by biopsy from 15 patients with chronic potassium deficiency. Four patients had three or more clinical episodes of acute pyelonephritis. Seven patients had two attacks of acute pyelonephritis. Three patients had single episodes of acute pyelonephritis. Proteinuria was noted in all patients. Twelve patients had abnormal numbers of leucocytes in the urinary sediment, but white blood cell casts were seen in only five instances. *E. coli* was cultured from the urine in five patients at the time of renal biopsy or within two days after the biopsy.

Quantitative morphological study of the renal tissue revealed "chronic pyelonephritis" in nine patients and interstitial fibrosis in five. Hydronephrosis was found in one by pyelography. Only one patient had hydropic vacuolar degeneration of the proximal convolutions of tubules. Electronmicroscopic study of the tissue revealed large hydropic vacuoles in the proximal convolutions and abnormal swollen mitochondria with decreased numbers of cristae within the mitochondria.

The review of the literature on chronic potassium depletion with the clinical and experimental studies reported indicate that prolonged potassium deficiency produces irreversible renal lesions identical to the morphological lesions of "chronic pyelonephritis." Furthermore, these studies indicate that the human nephron requires adequate supplements of potassium to preserve its functional and structural integrity. Without adequate stores of potassium the kidney becomes prone to irreversible morphological changes.

Respiratory Support in the Postoperative Period. E. W. STAUB and E. J. BEATTIE, JR., *The Surgical Clinics of North America*, Vol. 44, No. 1, pp. 227-241, February, 1964.

With increased knowledge of pulmonary physiology, and the development of improved positive pressure respirators, it has become possible to guide the patient with significant pulmonary problems through an otherwise perilous postoperative period. Major factors leading to respiratory insufficiency during the postoperative period include: 1) underlying pulmonary disease; 2) extensive pulmonary resection, 3) postanesthetic central depression; 4) ventilation-perfusion imbalance; 5) incisional pain; 6) patient fatigue; and, 7) neuromuscular weakness.

Respiration consists of three phases: ventilation, perfusion, and diffusion. The defects commonly encountered postoperatively are those of depressed ventilation (hypoventila-

tion), and ventilation-perfusion imbalance (atelectasis and/or pneumonia). Carbon dioxide retention and/or hypoxemia result in respiratory and metabolic acidosis, and constitute the primary indication for the use of tracheostomy and assisted ventilation. Prophylactic tracheostomy with planned postoperative assistance is suggested for patients who have minimal pulmonary reserve by preoperative evaluation, and in patients with marked pulmonary hypertension. The types of respirators available are pressure-cycled and volume-cycled. Meticulous care of the patient and his airway is essential to success.

Ancillary Procedures in Hand Surgery. J. H. SCHNEEWIND, *The Surgical Clinics of North America*, Vol. 44, No. 1, pp. 263-271, February, 1964.

Use of the Tourniquet—The use of the tourniquet to afford a bloodless field during operations on the hand is an accepted practice and is most valuable as it allows precise dissection of nerves, blood vessels and tendons without the complication of troublesome bleeding and oozing. Because of the danger of tourniquet palsy following prolonged compression, the time factor must be regulated very carefully.

Regional Nerve Block—There are distinct advantages to the use of either axillary or wrist nerve block in upper extremity surgery. Injuries usually require immediate care and the patient is often unprepared for a general anesthetic.

Compression Dressings and Splints—The application of a bulky compression dressing following operation or injury minimizes swelling and when used with proper splints puts injured tissues at rest (Fig. 1).

Additional ancillary procedures in surgery of the hand include electromyography, the finger printing test and physical and occupational therapy.

Isolation and Partial Characterization of Transferrin from Normal Human Plasma. A. BEZKOROVAINY, M. E. RAFELSON, JR. and V. LIKHTE, *Archives of Biochemistry and Biophysics*, Vol. 103, No. 3, pp. 371-378, December, 1963.

Transferrin of the C-4 type was prepared from human plasma by a method involving precipitation with ammonium sulfate and chromatography on DEAE-cellulose.

Physical and chemical properties of this preparation were in good agreement with those documented in literature. Molecular weight of transferrin, determined by the sedimentation equilibrium method, was $93,000 \pm 3000$, the isoelectric point was at about pH 4.9, and the sedimentation constant at infinite dilution was 5.64 at the isoelectric point.

The Oliguric Surgical Patient. J. M. SOMERNDIKE, W. E. WATKINS, and S. G. ECONOMOU, *The Surgical Clinics of North America*, Vol. 44, No. 1, pp. 291-298, February 1964.

Oliguria in the surgical patient is not an uncommon contributing factor in postoperative morbidity and mortality. Its appearance demands instant attention and prompt action directed toward its reversal. The concept of extrarenal failure has been presented and its relations to true renal failure emphasized. Differentiating characteristics have been noted for each, specific etiologies discussed in light of the disordered physiology, diagnostic aids suggested, and a program of management outlined.

The definitive care of the patient with renal failure is largely the province of the medical center with the proper experience and facilities. Specialized units with trained personnel and available extensive laboratory aids are available throughout the country and should be utilized. The condition of the patient on arrival at the treatment facility and the eventual outcome may be critically influenced by the early initiation of measures to obtain proper fluid balance and adequate nutrition, and by the accurate recording of baseline controls. Proper appreciation of the pathophysiology involved is essential for the application of effective prophylaxis and treatment of this problem. A clear understanding of this is the responsibility of every physician who undertakes the care of a surgical patient.

The Role of Drainage Following Biliary Tract Surgery. E. LEE STROHL, W. G. DIFFENBAUGH, and R. E. ANDERSON, *Surgical Clinics of North America*, Vol. 44, No. 1, pp. 281-291, February, 1964.

Most surgeons are thoroughly convinced, from experience and observation, that it is preferable to carry out drainage after biliary tract surgery and achieve a good result than omit a drain and risk the accumulation of bile, blood, or peritoneal fluid within the abdominal cavity. A drain, however, should never be a substitute for cautious and meticulous surgery.

Bile peritonitis is a catastrophe which may follow accidental or surgical trauma to the biliary tract. Bile leaking from the wound, after operation, may be due to injury of an accessory duct rather than improper ligation of the cystic duct. The leakage will occur in the simplest procedure when the liver is dislocated out of its bed, by rotation. Short accessory ducts may be torn by this maneuver. In addition, injury to an aberrant duct, unknowingly, is a hazard in every patient. Some of the aberrant ducts are very narrow in caliber, and may be injured during cholecystectomy.

The irritating properties of bile, and its ability to inhibit normal physiological defense, tend to produce severe peritoneal reaction which, if untreated, terminates in death in one-half to three quarters of patients. Acute and sub-acute stages of the disease are treated by immediate drainage and irrigation of the peritoneal cavity. The shock and dehydration syndromes that accompany the early phases of bile peritonitis must be corrected promptly. Antibiotic drugs should be used to combat infection.

In our opinion, whenever the biliary tract is approached surgically, the serious consequences of bile peritonitis can be avoided by the use of drains.

Newer Anticancer Agents. F. E. KNOCK, *Medical Clinics of North America*, Vol. 48, No. 2, p. 106, March, 1964.

Anticancer agents in clinical use attack especially nucleic acids or associated nuclear protein at the level of either monomers or polymers. The most frequently used drugs comprise two large classes chemically: (1) fraudulent monomers or antimetabolites, like 5-FU or methotrexate; and (2) drugs with significant activity against functional groups on cellular polymers, such as alkylating agents and thiolprives. Particularly for drugs attacking functional groups on cellular polymers, profound influence by intimate submicroscopic chemical milieu on reactions of functional groups in polymers necessitates precise chemical matching of anticancer drugs to target polymers in the patient's own cancer cells.

Antimetabolites; alkylating agents and thiolprives; natural products (alkaloids and antibiotics); steroids and miscellaneous compounds in current clinical use are reviewed, as well as immunochemical and physical agents.

Many potentially lethal drugs are now available for cancer chemotherapy from the above groups. Blind use of powerful chemical tools has no future for this field or any other. The enormous chemical heterogeneity of histologically similar tumors must be recognized to dominate the problems of clinical cancer chemotherapy. Significant progress can be anticipated when total cancer therapy for each patient is individualized in accord with the chemical reactivities of the patient's own cancer cells.

Studies on the Neuraminidase of Influenza Virus. II. Additional Properties of the Enzymes from the Asian and PR 8 Strains. M. E. RAFELSON, JR., M. SCHNEIR and V. W. WILSON, JR., *Archives of Biochemistry and Biophysics*, Vol. 103, No. 3, pp. 424-430, December, 1963.

A comparison of the properties of purified neuraminidases from the Asian and PR 8 strains of influenza virus has shown a number of differences. The enzymes differ in their K_M values for N-acetylneuraminylactose, in their K_I values for the competitive inhibitor N-acetylneuraminic acid, in heat stability, and in their pH optimum for a number of substrates. Preliminary studies indicate that the two enzymes are immunologically distinguishable, and the degree of neutralization of enzyme action by specific antiserum is dependent on the substrate employed.

TABLE OF CONTENTS

<i>page</i>	
82.....	In Defense of Gout: The Importance of Uric Acid in Diagnosis EVAN M. BARTON, M.D., ROBERT E. DEDMON, M.D. AND WALTER R. SUNDSTROM, M.D.
94.....	Systemic Lupus Erythematosus: The Failure of Urinalysis and Simple Renal Function Tests to Predict Reliably the Underlying Renal Pathology VICTOR E. POLLAK, M.D., FRANKLIN D. SCHWARTZ, M.D. AND CONRAD L. PIRANI, M.D.
104.....	Contrast Arthrography of the Knee Joint RICHARD E. BUENGER, M.D.
109.....	The Temporo-Mandibular Joints in Rheumatoid Arthritis MAURICE L. BOGDONOFF, M.D.
112.....	Adverse Effects of Drug Therapy in Rheumatoid Arthritis GEORGE W. STUPPY, M.D.
119.....	Rheumatoid Arthritis and Orthopaedic Surgery WILLIAM F. HEJNA, M.D.
126.....	Aseptic Necrosis of Bone Complicating Corticosteroid Therapy for Pemphigus Vulgaris JAMES C. LOWE II, M.D.
132.....	Cytotoxic Antibodies: Their Significance in "Autoimmune" Disease ROBERT E. DEDMON, M.D.

IN DEFENSE OF GOUT:

The Importance of Uric Acid in Diagnosis

EVAN M. BARTON, M.D.¹

ROBERT E. DEDMON, M.D.²

WALTER R. SUNDSTROM, M.D.³

From a neglected disease gout has become in the past twenty-five years one of the more commonly diagnosed arthritic afflictions. Once a status symbol of maleness, of gentle or royal birth^{1,2} and even of intellectual capacity, the diagnosis now seems to be more liberally applied. It is true that "poor man's gout" has long been with us. One of us recalls a series of such patients derived from the Central Free Dispensary reported before a group attending Rush Medical College Alumni Clinics *ca* 1939 (Fig. 1).³ Also, there is no dearth of severe tophaceous gout in certain Veterans Administration Hospital populations, a circumstance which has attracted the notice of surgeons.^{4,5}

But does gout occur other than rarely in women? Is there so-called atypical gout, a grumbling somewhat non-specific painful state without classical acute episodes? Can early gout affect the spine alone? What about the patient with obvious rheumatoid disease whose arthritis is presumed to have additionally a "gouty element;" can rheumatoid arthritis and gout coexist? These questions are not infrequently posed in ordinary medical or orthopedic conversation, and have even reached print.^{6,7,8} We believe that an affirmative answer, though per-

haps fashionable, or even flattering to patients, or welcomed by them, is likely to be incorrect. The pendulum in such cases has swung too far, and there tends to be overdiagnosis of gout.

It follows that reliable means must be offered to settle individual clinical problems. Clinical experience, as Hippocrates may have meant, is fallacious⁹ and is subject to bias. However, classical podagra is among the best documented clinical phenomena in medicine. When seen it is all but unmistakable.* Yet it remains of importance to the patient that the physician clinch the correct diagnosis as early and as securely as possible since the implications of lifelong hyperuricemia are far from frivolous. This is all the more true if the diagnosis of gout in a particular patient is at all questionable.

In the early stages of gout a clinical diagnosis is suggested by history or observation of characteristic acute recurrent attacks responsive to adequate colchicine therapy. In later stages, when the arthritis is chronic and tophaceous, the physical appearances, including roentgenography, may be all but patho-

From the Division of Medicine, Presbyterian-St. Luke's Hospital, and the Medical Service, Veterans Administration Hospital, Hines, Illinois

¹ Attending Physician, Presbyterian-St. Luke's Hospital and Hines Veterans Administration Hospital; Clinical Professor of Medicine (Rush), University of Illinois College of Medicine

² Adjunct Physician, Presbyterian-St. Luke's Hospital; Instructor in Medicine; University of Illinois College of Medicine. Present Address—211 N. Commercial Street, Neenah, Wisconsin

³ Resident in Medicine, Hines Veterans Administration Hospital; Teaching Fellow, Stritch School of Medicine, Loyola University

*(The authors do not include in this article a description of classical gout, either acute or chronic, since such is readily available, and by more facile pens than ours.)



Fig. 1.—Severe gouty deformity in hands of a 40 year old man on relief (1939). Duration of arthritis, 18½ years. He died some 5 years later at Cook County Hospital of hypertensive cerebrovascular disease.

gnomonic. Nevertheless, in all cases early and late it is requisite to implicate the central figure or villain of the piece, uric acid. Three methods are readily available. First, sodium urate crystals may be identified in synovial fluid removed from acutely inflamed gouty joints. Second is the demonstration of hyperuricemia. A third method is the histopathologic observation of urate crystals in tissue sections. We propose to examine the question of colchicine response, to discuss these diagnostic methods and present illustrative clinical material.

Therapeutic Response to Colchicine

If this drug is given early in the course of an acute attack of gout, moderate improvement occurs within 12 hours and marked improvement in 24 hours. This requires adequate dosage and prompt institution of treatment.¹⁰ In our experi-

ence, 3 to 4 mg in divided doses over 6 to 8 hours may suffice initially. The response to colchicine has been regarded as sufficiently specific in gout to serve as a diagnostic test. Difficulty occurs when colchicine is not given early enough *in the acute attack* or if the dose is inadequate. The concomitant use of corticotrophin, steroids or phenylbutazone nullifies the test as a diagnostic procedure. Questions about whether colchicine is, in a particular instance, absorbed may be answered by using it intravenously. It is worth emphasizing that, while some relief may occur with colchicine during the first few hours of oral administration, as much as 8 mg. may be required to abort the attack and this may require 24 hours or more.

The usefulness of the response of colchicine as a diagnostic test has been challenged by Kaplan¹¹ and Zuckner.¹² Kaplan has noted a response in patients with sarcoid arthritis and Zuckner found

a response to orally administered colchicine in 10 of 30 patients with rheumatoid arthritis. The latter author found no difference when a placebo was used in one-half of the patients who responded. Both subjective improvement and objective findings such as changes in swelling, heat, tenderness, and range of motion were evaluated. The problem here is partly one of interpretation. The particular rheumatoid joint or joints used to judge improvement by must be severely and acutely enough involved to allow a sharp end-point in estimating "improvement." Further, there is no accurate way of determining dose response unless the drug is given intravenously. For these reasons it does not seem valid on the basis of Zuckner's observations to put aside the long established concept of specificity of colchicine in gout or to detract from its proper use as a diagnostic test.

Until very recently the mechanism of action of colchicine in terminating attacks of gout has defied explanation and has been considered unrelated to the uric acid problem. However, Seegmiller and his coworkers have proposed an attractive hypothesis.¹³ They hold that colchicine acts by inhibiting local phagocytosis of urate crystals in acute gouty synovitis and thus a vicious cycle of changes is broken. Synovial fluid pH is no longer critically reduced and hence further crystalline material is not precipitated and the attack ends. This concept derives from observations on crystalline material in joints both experimentally and clinically.

The diagnostic value of urate crystals in joint fluid is discussed next.

Urate Crystals in Synovial Fluid

Synovial fluid aspiration has been practiced for many years in the diagnosis and relief of acute arthritic effusions. Since 1961, the procedure has been used as an accurate method of demonstrating sodium urate crystals in gouty joints.¹⁴ Indeed, the implications of the regular presence of such microcrystals have led to important concepts concerning the

true nature of the acute inflammatory lesions of gout and the genesis of the acute attack.¹⁵ As a diagnostic procedure, the method is rapid and safe. It depends on the demonstration by polarized light microscopy of birefringent needle shaped crystals in the aspirated synovial fluid. After removal under aseptic precautions from the inflamed joint, a drop of the fluid is placed on a glass slide under a coverslip. Ordinary light microscopy permits observation and differential counting of leukocytes. Crystals may be seen and are then further viewed under polarized light. Sodium urate crystals appear as bright birefringent needles with parallel sides. Lying free they have sharp pointed ends. Seen within phagocytes, the ends appear rounded. (Fig. 2) In the effusions of degenerative joint disease fibrillar strands may cause confusion. However, they have a less regular structure, the sides are not parallel, ends are frayed and they are not birefringent.

Table I indicates frequency of demonstration of synovial fluid crystals in patients with gout, in our own small series. Our controls consisted of patients with rheumatoid arthritis, osteoarthritis and effusions secondary to trauma. No crystalline structures have been found in the control group. Our results correspond to the larger series reported by McCarty¹⁶ and Zvaifler.¹⁷

In chondrocalcinosis or "pseudogout," a condition which may be more common than supposed, crystals may appear in joint fluid which resemble sodium urate.¹⁸ In one form they are needle shaped. Appropriate studies have shown that they are hydrated calcium pyrophosphate. The condition might at times be confused with gout on clinical grounds. However, distinction should readily be made roentgenographically, as changes seen on x-ray in the pseudogout syndrome are said to be unique, while the arthritic manifestations do not respond dramatically, if at all, to colchicine. In joint fluid examination, urate crystals may be dissolved upon addition of uricase, whereas calcium pyrophosphate crystals are not.



Fig. 2.—Wet preparation of sodium urate crystals removed from acutely inflamed knee joint, viewed by polarized light (X 43).

SERUM URIC ACID

Methods of Assay

Serum uric acid is most commonly determined by a colorimetric procedure which involves the reduction of phosphotungstic acid to a colored product

by uric acid. The determination is carried out on a protein-free filtrate of serum. A number of modifications of Folin's original procedure¹⁹ have been employed, notably those devised by Archibald²⁰ and Henry et al.²¹ In the Presbyterian-St. Luke's Hospital clinical laboratory

TABLE I
DEMONSTRATION OF URATE CRYSTALS IN SYNOVIAL FLUID
26 GOUTY PATIENTS

ASPIRATIONS	
Total.....	28
Crystals found.....	25
Crystals not found.....	3
Crystals within leukocytes.....	21
JOINTS INVOLVED	
Total.....	28
1st Metatarsophalangeal.....	15
Knee.....	7
Ankle.....	3
Hip, wrist, 2nd metacarpophalangeal.....	1 each

the latter method is used with slight modification.²² In this procedure serum proteins are precipitated by sodium tungstate in the presence of sulfuric acid and color development is carried out in alkaline solution with phosphotungstate in the presence of sodium carbonate.

A more specific method is the enzymatic spectrophotometric procedure of Kalckar²³ as modified by Praetorius²⁴ and others.²⁵ In this procedure serum is diluted in alkaline buffer and its absorption is determined at 292 mμ in a spectrophotometer before and after the addition of uricase. Uricase is highly specific and in alkaline solution converts uric acid to allantoin which does not absorb at this wave length. The change in absorption before and after uricase treatment thereby provides determination of the amount of uric acid present.²⁶

There are several errors which may be operative when serum and urinary uric acid are determined by colorimetric procedures.^{26,27} Serum salicylate values of greater than 13 mg. per cent may result in falsely elevated values,²⁷ as may hyperglycemia,²⁸ and the presence in serum of caffeine and theophylline.²⁶ Also, urinary uric acid determination may be erroneously elevated owing to the presence of metabolites of salicylate such as gentisic acid.²⁷ Removal of serum proteins causes some loss of uric acid because of co-precipitation, but this error is usually cancelled by non-urate reducing substances present in biological material. False high values resulting from salicylate in serum ordinarily do not result in erroneous diagnosis because of the uricosuric effect of high doses of salicylate.²⁷

The Normal Range of Serum Uric Acid

The point in question is how to define the upper limits of normal. The influence of method variability, age, sex, drugs, other diseases, and distribution in various populations must all be considered. Traditionally, and indeed somewhat arbitrarily, 6 mg. per cent has been considered the upper limit of normal in males when colorimetric procedures are

employed in the estimation of serum uric acid.²⁹ A level of 5.5 mg. per cent applies to females. When the enzymatic method is employed levels of 7.0 mg. per cent³⁰ and 7.5 mg. per cent³¹ for males and 6.0 mg. per cent³⁰ and 5.7 mg. per cent³¹ for females are suggested.

Most reported normal ranges have included only one standard deviation and therefore include only about 67 per cent of normal individuals. If one uses 95 per cent confidence limits the colorimetric procedure of Henry et al²¹ gives upper limits of normal of 7.0 mg. per cent for males and 5.7 mg. per cent for females.³² In the Presbyterian-St. Luke's Hospital laboratory values of 6.2 and 5.5 mg. per cent are offered as upper limits of normal for males and females respectively, but data for statistical treatment are not available,²² and we are inclined to regard levels of above 7.0 in males and 6.0 mg. per cent in females as potentially abnormal with the colorimetric procedure. In one series²⁹ a single value of 7.2 mg. per cent in a female was considered potentially normal or abnormal. More than one determination is often desirable.

All that one can say on the basis of the available data is that interpretation of serum uric acid levels as generally estimated is at times difficult and that previously employed upper limits of normal are probably too low. Values are slightly higher with the enzymatic than with the colorimetric procedures.

Hyperuricemia

In spite of problems in methodology and in deciding upon the upper limits of normal for serum uric acid, hyperuricemia remains one of the major criteria in the diagnosis of gout.³³ However, hyperuricemia has been found in a variety of other circumstances, and in some of these clinical gout may result. Such is the case with hyperuricemia associated with leukemia, polycythemia vera, psoriasis, administration of chlorothiazide, hyperlacticacidemia of glycogen storage disease, and chronic renal disease.^{30,34-42} Table II lists various conditions in which hyperuricemia has been reported.

TABLE II
CONDITIONS WITH ASSOCIATED HYPERURICEMIA
(26, 27, 30, 34-51)

1. Normal population (about 10 per cent)	
2. Primary metabolic gout	
3. Hematologic disorders (e.g. leukemia, polycythemia, pernicious anemia during treatment, hemolytic anemia, multiple myeloma)	
4. Other metabolic diseases:	
A. Obesity	E. Acromegaly
B. Diabetic Mellitus	F. Hyperparathyroidism
C. Hyperthyroidism	G. Hypoparathyroidism
D. Hypothyroidism	H. Glycogen Storage Disease
5. Chronic Renal Disease with Azotemia	
6. Psoriasis (also psoriasis associated with sarcoidosis) ⁵⁰	
7. Febrile infections (e.g. resolving pneumonia)	
8. Poisoning (e.g. lead, carbon monoxide, methanol, ammonia, barbiturates)	
9. Toxemia of pregnancy	
10. Lactate infusion	
11. Muscular exercise	
12. Starvation	
13. Rheumatoid Arthritis*	
14. Drugs (salicylates in low dosage, chlorothiazide, pyrazinamide, chlorthalidone, acetazolamide)	
15. Adrenalectomy for hypertension (not related to blood pressure level, drugs, or renal failure)	

*This has been challenged by Weaver and Smyth²⁹

About 95 per cent of patients with gout have hyperuricemia^{30,43,44} and the level of serum uric acid is usually between 8 and 12 mg. per cent in untreated patients with primary gout.³⁰ A confirmed value of greater than 15 mg. per cent requires a careful search for another disease (e.g. leukemia). In view of the common occurrence of urate retention in chronic renal disease, simultaneous determination of the blood urea nitrogen and serum uric acid should be employed in screening for hyperuricemia. Dietary purine restriction for 2 to 3 days will lower the serum uric acid level about 1 mg. per cent.³¹

Urate Crystals in Tissue (Tophi)

The diagnostic value of accessible tophi is greatly enhanced if these accumulations, visible or palpable, can be shown to be made up of urate deposits. First of all, the classical tophus in the ear, even when its chalky content is not obvious, may be distinguished clinically from other similar lumps by its opacity to transmitted light as from the small beam of a pen sized flashlight held against the ear. This opacity seems to be

a characteristic of urate deposits and may be noted grossly also in thin histologic sections, stained or unstained, of tophaceous material. Customarily, ear tophi are teased with a needle, the suspected material placed on a slide and examined as described for synovial fluid.

Juxta-articular tophi are often available for biopsy, commonly about the elbows. We have seen and proved the gouty nature of one such on the front of the right patella in a man who habitually struck this knee as he knelt in church. The nodular masses often feel rubbery and may be mistaken for rheumatoid nodules unless their tophaceous nature is established by histologic examination. Care must be taken that suspected material be fixed in absolute alcohol in order to preserve the urate crystals in situ. The De Galantha method⁵² stains them black with silver. Proper fixation is important diagnostically, for although the granuloma of gout is often characteristic in formalin fixed sections from which the crystals have been lost in preparation, the conspicuous and often beautifully arranged crystals in alcohol fixed material constitute more convinc-

ing evidence. The method also enables one to observe crystal deposition in early lesions where urates have not as yet excited the mature granulomatous response.

Occasionally whole digits may be examined in this way, as in the case of the woman reported by Traut et al (53). Her finger, extensively deformed and rendered useless by tophaceous masses, was amputated; thus providing a sceptical clinician with irrefutable evidence that gout can occur in a female. Poske (54) has reported a 39 year old Filipino man, native of Luzon, whose back stiffness, coupled with x-ray alterations in sacroiliac joints, led to a diagnosis of rheumatoid spondylitis for which the patient received roentgen therapy. As the disease progressed, severe tophaceous involvement occurred in peripheral joints, elbow bursae and elsewhere. A biopsy of the right sacroiliac joint fixed in alcohol and stained by the De Galantha method demonstrated urate crystals. (Figs. 3 & 4)*

Illustrative Case Reports

The value of some of these diagnostic methods as well as their limitations may be further illustrated by the following case reports.

Case Abstract 1

Mrs. K. D. (697570) P.StL.H. No., a 49 year old white female business executive was admitted to Presbyterian-St. Luke's Hospital with a history of severe pain and swelling in both feet and ankles for four days. One month before, transient pain had been noted in the same areas without other symptoms or disability. In the current attack pain began suddenly in the dorsum of the right foot and ankle and was followed by pain and swelling in the dorsum of the left foot and ankle. The pain increased in severity during the next 48 hours so that she could not walk. There was no pain in either great toe. She had no history of renal disease or hypertension. She had previously been treated for thrombo-



Fig. 3.—Photomicrograph of urate crystals in sacroiliac joint biopsy (DeGalantha stain).

*Members of the Chicago Rheumatism Society had the opportunity to observe this man in 1949 and again in 1952 (54, 55).



Fig. 4.—Photomicrograph of alcohol fixed urate crystals in biopsy of olecranon bursa. Same patient as Fig. 3.

phlebitis with dicoumarol. Menstrual periods had been regular the last having occurred three weeks before admission. Two sisters, one brother and one nephew were said to be afflicted with gout. One other brother had arthritis of unknown type.

Physical examination revealed temperature of 99.4 degrees orally and a blood pressure of 140/80. The tops of both feet were swollen, red, and hot, and gave the appearance of cellulitis. There was slight tenderness and swelling of the left ankle. The metatarsophalangeal joints were normal. There were

no tophi or subcutaneous nodules. The remaining joints were normal. The clinical diagnosis was gout.

Laboratory data revealed a hemoglobin of 12.8 grams per cent and a WBC of 14,080. Urinalysis was normal. Sedimentation rate was 23 mm/hour (Wintrobe). Serum uric acid on admission was 10.7 mg. per cent and the blood urea nitrogen was 13 mg. per cent. Subsequent serum uric acid determinations revealed levels of 8.8, 7.1, 5.3, and 5.0 mg. per cent during her course of therapy. X-ray studies of the feet and ankles were normal.

The patient received colchicine, ACTH, and finally probenecid. She responded to treatment and was discharged on a combination of colchicine and probenecid. She has had no recurrence of her attacks on this maintenance therapy during the past 2 years.

The diagnosis of gout in this case depends on the strong family history and the elevated levels of uric acid in her serum. The effect of treatment must be called nonspecific since ACTH was used. Within these limitations, one is justified in assuming that this woman represents that rarity, gout in a female prior to the menopause, though proof would demand demonstration of urate crystals in inflammatory synovial fluid.

Case Abstract 2

Mr. J. S. P.StL.H.No., (021696) a 67 year old Negro male, was admitted to Presbyterian-St. Luke's Hospital for removal of a cataract. He had been followed in the outpatient clinic for hypertension and had received phenobarbital, reserpine and chlorothiazide. The patient had complained of generalized joint pains intermittently for several years, and fluid had been withdrawn from a swollen knee on one occasion. Ten days prior to admission the patient had an attack of multiple joint pains without objective physical findings. There was no family history of gout. Blood for a serum uric acid was drawn, but the report did not reach the chart prior to his admission for cataract surgery. (The serum uric acid was 10.0 mg. per cent!) The pains subsided without treatment, the patient was admitted and surgery was performed. Two days following operation his left knee and ankle became acutely tender, hot, and swollen. The toes were not involved and all other joints were normal. His temperature was 100.2 degrees orally. The serum uric acid then was 10.8 mg. per cent and the blood urea nitrogen, 26 mg. per cent.

Aspiration of the left knee yielded 20 ml. of clear, yellow fluid. The addition of acetic acid to a portion of the fluid

produced a normal ropy mucin clot. A smear of the fluid showed over 90 per cent polymorphonuclear leukocytes, and examination with polarized light revealed the presence of birefringent needle-like crystals, many within polymorphonuclear leukocytes. Cultures of the fluid were negative. Following oral colchicine the patient had marked improvement of his symptoms in 48 hours. Probenecid was also prescribed. The chlorothiazide was omitted from his antihypertensive regime. Serum uric acid values during convalescence and under the influence of probenecid were successively 9.7, 8.2, 7.2, 6.5, and 6.1 mg. per cent by the time of discharge.

The frank gouty attack in this man was provoked by cataract surgery in the classical postoperative pattern. Preexisting hyperuricemia may well have been aggravated by chlorothiazide administration, setting the stage for the acute attack during which characteristic sodium urate crystals were demonstrated in synovial fluid. It is possible that the nonspecific joint complaints of prior years were gout. There is insufficient evidence to decide the point. But the acute arthralgia 10 days before surgery seems to have been a specific herald of the full blown disease.

Case Abstract 3

H.K.R. PStLH.No. (642175), a farmer now 54 years old, a patient of Dr. Richard B. Capps, had had episodes of acute joint swelling for over 25 years, occurring 3 to 4 times a year in toes, ankles, wrists and fingers. Attacks lasted 12 to 24 hours and were relieved by colchicine. In 1942, a nodule near the right elbow had been removed in another city and had been reported to contain urate crystals. Tophi had been noted in both ears; these regressed and disappeared following institution of therapy with probenecid in 1956. Uric acid values had been determined on whole blood and ranged from 1.6 mg./100 to 6.1. The latter value was found on February 18, 1955, and of 8 determinations in the next 5 years none exceeded

4.3. Nodules had recurred at the right elbow and appeared near the left elbow also, and the patient spoke of them as "tophi." Frequency of acute attacks had decreased. In May, 1959, he had "Asiatic flu." This was followed by bilateral pleural effusion, a continuing low grade fever, dyspnea, and increased joint swelling noted particularly in the knees.

When examined in October, 1959, there was evidence of destructive arthritis in shoulders and elbows. Knees were warm and swollen with fluid. Ankles and feet showed periarticular thickening and tenderness. Many finger joints were chronically swollen. The left middle finger had recently acquired a typical "swan neck" deformity. Firm, rubbery juxtaarticular nodules were present at each elbow, larger on the left.

Serum uric acid was 3 mg./100. There was a mild anemia. Erythrocyte sedimentation rate was 36 mm. (Wintrobe). Kahn was negative. Sensitized sheep cell agglutination was positive in a titer of 1:256 and latex fixation was positive 1:1024. Four LE cell preparations were negative. Bone marrow contained increased stainable iron; myeloid and erythroid maturation was normal. Biopsy of skin and muscle of left calf was normal. Both knees were injected with hydrocortisone with temporary improvement. There was no change in his basic regimen.

He was readmitted January 5, 1963, because of deterioration in general strength. His gait had become a shuffle and he had difficulty dressing and undressing without help. There had been no acute flares of arthritis and no recurrence of the pleurisy. Serum uric acid was 5.5 mg./100; 2 LE cell preparations were negative. Sheep cell agglutination titer was 1:64; latex fixation titer, 1:1280.

All this man's x-ray material on file was reviewed. There were punched out areas in metatarsophalangeal joints, more on the right, "typical of advanced gout." Small pseudocysts were also seen in these joints compatible with rheumatoid arthritis. In the knees there were punched out areas of lucency, and bone eburnation at medial plateaus. In elbows, cartilage space was narrowed with irregularities in subchondral bone compatible with late rheumatoid arthritis. Dr. M. Bogdonoff wrote: "The radiologic appearance of many parts of the skeleton of this patient is confusing. There are features of both gout and rheumatoid arthritis present."

On 1-18-63 the juxta-articular nodule at the right elbow was removed for biopsy. When cut in the operating room it contained white crystalline material grossly. On histologic examination the changes were typical of a rheumatoid nodule having lime salts in its interior. Suitably prepared sections failed to show any urate deposit or morphologic changes of gout.

It seems likely that this patient suffered for many years with gout. The long history, including colchicine response, is that of gout and one is impressed with the lessening numbers of acute attacks and the disappearance of ear tophi after uricosuric therapy was given. About 1959, however, following pneumonitis and pleurisy, the character of the arthritis changed, becoming active, smoldering, intractable and disabling, with clinical, serologic, roentgenographic and finally in 1963 unequivocal histologic evidence of rheumatoid disease. There was no evidence then for hyperuricemia and the patient's management became that of chronic rheumatoid arthritis.

SUMMARY

The correct diagnosis of gout requires precision in the use of clinical and laboratory procedures. Among the former, the therapeutic test with colchicine remains dependable and valid when

properly used. Identification of sodium urate crystals in inflammatory joint fluid is a simple and rewarding diagnostic procedure, as is also the identification of urates in tophi. Both are pathognomic

for gout and deserve more frequent use.

The normal upper limit of serum uric acid values varies with the method used and is hard to define. A useful figure for males above which hyperuricemia may initially be assumed is 7 mg. per cent by colorimetric methods in common use. The corresponding value for women is about 1mg percent lower. Hyperuricemia when thus defined occurs in some normal people, in almost everyone with

primary gout, and in persons exhibiting a large variety of syndromes many of which are associated with secondary gouty arthritis.

The appreciation of the leading role of uric acid and crystalline urates in gout will tend to make its diagnosis more precise. On the one hand this will increase awareness of the many clinical constellations in which gout may appear, and on the other, prevent its overdiagnosis.



REFERENCES

1. Vallot, D'Aquin and Fagot.: *Journal de la Santé du Roi Louis XIV*, J.-A. Le Roi, ed., Paris, Auguste Durand, 1862.
2. Bywaters, E. G. L.: Gout in the Time and Person of George IV; A Case History. *Ann. Rheum. Dis.*, 21:325, 1962.
3. Barton, E. M.: Gout in the Indigent. Unpublished.
4. Larmon, W. A. and Kurtz, J. F.: The Surgical Management of Chronic Tophaceous Gout. *J. Bone and Joint Surg.*, 40-A:743, 1958.
5. Straub, L. R., Smith, J. W., Carpenter, G. K. and Dietz, G. H.: The Surgery of Gout in the Upper Extremity. *J. Bone and Joint Surg.*, 43-A:731, 1961.
6. Turner, R. E., Frank, M. J., van Ansdal, D. and Bollet, A. J.: Some Aspects of the Epidemiology of Gout, Sex and Race Incidenc. *Arch. Ind. Med.*, 106:400, 1960.
7. Lapidus, P. W. and Guidotti, F. P.: Gout in Orthopedic Practice. Review of 232 Cases. *Clinical Orthopaedics and Related Research*, 28:97, 1963.
8. Steindler, A.: On Atypical Gout. *Bull. Hosp. for Joint Diseases*, 12:404, October, 1951.
9. The genuine works of Hippocrates, tr. Francis Adams, London, Sydenham Society, 1849. Vol. 2, p. 697.
10. Bauer, W. and Singh, M. M.: Management of Gout. *New Eng. J. Med.*, 256: 171, 1957.
11. Kaplan, H.: Sarcoid Arthritis. *Arch. Int. Med.*, 112: 924, 1963.
12. Zuckner, J.: Responses to Colchicine Therapeutic Trail in Rheumatoid Arthritis. *New Eng. J. Med.*, 267:682, 1962.
13. Seegmiller, J. E. and Howell, R. R.: The Old and New Concepts of Acute Gouty Arthritis. *Arth. and Rheumat.* 5:616, 1962.

14. McCarty, D. J., Jr.: The Diagnosis of Gouty Arthritis: 18 Months Experience with a Pathognomonic Test. *Postgrad. Med.*, 33:142, 1963.
15. Seegmiller, J. E., Howell, R. R., Malawista, S. E.: The Inflammatory Reaction to Sodium Urate. *J.A.M.A.*, 180:469, 1962.
16. McCarty, D. J. and Hollander, J. L.: Identification of Urate Crystals in Gouty Synovial Fluid. *Ann. Int. Med.*, 54:452, 1961.
17. Zvaifler, N. J. and Pekin, T. J.: Significance of Urate Crystals in Synovial Fluid. *Arch. Int. Med.*, 111:99, 1963.
18. McCarty, D. J., Jr., and Gatter, R. A.: Pseudogout Syndrome (Articular Chondrocalcinosis). *Bull. Rheum. Dis.*, 14: (No. 5) 331, 1964.
19. Folin, O.: Standardized Methods for the Determination of Uric Acid in Unlaked Blood and Urine. *J. Biol. Chem.*, 101:111, 1933.
20. Archibald, R. M.: Colorimetric Measurement of Uric Acid. *Clin. Chem.*, 3:102, 1957.
21. Henry, R. J., Sobel, C., and Kim, J.: A Modified Carbonate-phosphotungstate Method for the Determination of Uric Acid and Comparison with the Spectrophotometric Uricase Method. *Am. J. Clin. Path.*, 28:152, 1957.
22. Kachmar, J.: Personal Communication, 1964.
23. Kalckar, H. M.: Differential Spectrophotometry of Purine Compounds by Means of Specific Enzymes. I. Determination of Hydroxypurine Compounds. *J. Biol. Chem.*, 167:429, 1947.
24. Praetorius, E. and Poulsen, H.: Enzymatic Determination of Uric Acid by Ultraviolet Spectrophotometry. *Scand. J. Clin. and Lab. Invest.*, 5:273, 1953.
25. Liddle, L., Seegmiller, J. E. and Laster, L.: The Enzymatic Spectrophotometric Method for Determination of Uric Acid. *J. Lab. and Clin. Med.*, 54:903, 1959.
26. Seegmiller, J. E., Laster, L., and Howell, R. R.: Biochemistry of Uric Acid and its Relation to Gout. *New Eng. J. Med.*, 268:712, 764, 821, 1963.
27. Grayzel, A. I., Liddle, L., and Seegmiller, J. E.: Diagnostic Significance of Hyperuricemia in Arthritis. *New Eng. J. Med.*, 265:763, 1961.
28. Bien, E. J. and Troll, W.: Interference by Glucose in Quantitative Determination of Uric Acid. *Proc. Soc. Exp. Biol. and Med.*, 73:370, 1950.
29. Weaver, W. F. and Smyth, C. J.: Serum Urate in Degenerative Joint Disease and Rheumatoid Arthritis. *Arth. and Rheum.*, 6:372, 1963.
30. Sorensen, L. B.: Current Concepts of Gout and its Treatment. *Med. Clin. N. Am.*, 47:169, 1963.
31. Brochner-Mortensen, K.: Review of Diagnostic Criteria and Known Etiological Factors in Gout. In: Kellgren, J. H., Jeffrey, M. R., and Ball, J. ed., *The Epidemiology of Chronic Rheumatism*, Philadelphia, F. A. Davies, 1963, p. 140.
32. Emmerson, B. T., and Sandilands, P.: The Normal Range of Plasma Urate Levels. *Australas. Ann. Med.*, 12:46, 1963.
33. Kellgren, J. H.: Diagnostic Criteria for Population Studies. *Bull. Rheum. Dis.*, 13:291, 1963.
34. Sorensen, L. B.: The Pathogenesis of Gout. *Arch. Int. Med.*, 109:379, 1962.
35. Gutman, A. B.: Primary and Secondary Gout. *Ann. Int. Med.*, 39:1062, 1953.
36. Talbott, J. H.: Gout and Blood Dyscrasias. *Medicine*, 38:173, 1959.
37. Denman, A. M., Szur, L. and Ansell, B. M.: Joint Complaints in Polycythaemia Vera. *Ann. Rheum. Dis.*, 23:139, 1964.
38. Eisen, A. Z. and Seegmiller, J. E.: Uric Acid Metabolism in Psoriasis. *J. Clin. Invest.*, 40:1486, 1961.
39. Bryant, J. M., et al.: Hyperuricemia Induced by Administration of Chlorthalidone and Other Sulfonamide Diuretics. *Am. J. Med.*, 33:408, 1962.
40. Aronoff, A.: Acute Gouty Arthritis Precipitated by Chlorothiazide. *New Eng. J. Med.*, 262:767, 1960.
41. Ayvasian, J. H. and Ayvasian, L. F.: Study of Hyperuricemia Induced by Hydrochlorothiazide and Acetazolamide Separately and in Combination. *J. Clin. Invest.*, 40:1961, 1961.
42. Holling, H. E.: Gout and Glycogen Storage Disease. *Ann. Int. Med.*, 58:654, 1963.
43. Talbott, J. H.: *Gout*, 2nd Ed., New York, Grune and Stratton, 1957.
44. Wyngaarden, J. B.: Gout. In: Stanbury, J. B., Wyngaarden, J. B. and Fredrickson, D. S., eds., *The Metabolic Basis of Inherited Disease*. New York, Blakiston, 1960, p. 679.
45. Fuller, R. W., Luce, M. W. and Mertz, E. T.: Serum Uric Acid in Mongolism. *Science*, 137:868, 1962.
46. Murphy, R. and Shipman, K. H.: Hyperuricemia During Total Fasting. *Arch. Int. Med.*, 112:954, 1963.
47. Yü, T. F. and Gutman, A. B.: Study of Paradoxical Effects of Salicylate in Low, Intermediate and High Dosage on Renal Mechanisms for Excretion of Urate in Man. *J. Clin. Invest.*, 38:1298, 1959.
48. Padova, J. and Bendersky, G.: Hyperuricemia in Diabetic Ketoacidosis. *New Eng. J. Med.*, 267:530, 1962.
49. Sataline, L. R., Powell, C., and Hamwi, G. J.: Suppression of the Hypercalcemia of Thyrotoxicosis by Corticosteroids. *New Eng. J. Med.*, 267:646, 1962.
50. Kaplan, H. and Klatskin, G.: Sarcoidosis, Psoriasis, and Gout: Syndrome or Coincidence? *Yale J. Biol. and Med.*, 32:335, 1959.
51. Itskovitz, H. D. and Sellers, A. M.: Gout and Hyperuricemia after Adrenalectomy for Hypertension. *New Eng. J. Med.*, 268, 1105, 1963.
52. DeGalantha, E.: Technic for Preservation and Microscopic Demonstration of Nodules in Gout. *Am. J. Clin. Path.*, 5:165, 1935.
53. Traut, E. F., Kostal, F. and Volini, F.: Gout in Women. *Ill. Med. J.*, 125:147, 1964.
54. Poske, R. M.: Gout of the Sacroiliac Joints Simulating Rheumatoid Arthritis. *Medical Service Bull., Hines Veterans Administration Hospital, Fall, 1954*, p. 25.
55. Zivin, S., Black, A. and Barton, E. M.: Gout in a Filipino with Involvement of Sacroiliac Joints. 1949, unpublished.
56. Zivin, S. and Black, A.: Unusual Manifestation of Gout: Follow-Up Report, 1952. Unpublished.

SYSTEMIC LUPUS ERYTHEMATOSUS:

The Failure of Urinalysis and Simple Renal Function Tests to Predict Reliably the Underlying Renal Pathology

VICTOR E. POLLAK, M.D.¹

FRANKLIN D. SCHWARTZ, M.D.²

CONRAD L. PIRANI, M.D.³

INTRODUCTION

The clinical and histologic aspects of the renal involvement which occurs in systemic lupus erythematosus (SLE) have been described in previous papers from this laboratory^{1,2,3}. In 1957, serial observations were presented on 31 patients ill with SLE who had been followed for a maximum of two years after they were first studied¹. Recently we have reported on the clinical and histologic aspects of the natural history of the renal involvement in 87 patients with SLE who were followed after the initial renal biopsy for periods of time varying from seven months to eight years².

It was found that the kidneys in patients with SLE may be normal histologically or that there may be evidence of lupus glomerulitis, membranous lupus glomerulonephritis or active lupus glomerulonephritis². Of the three abnormal histologic patterns only active lupus glomerulonephritis was associated with

rapidly progressive deterioration of renal function and death in renal failure. We^{2,3} and others^{4,5,6} have observed that the natural history of active lupus glomerulonephritis could be modified by the use of large doses of prednisone given deliberately for a period of six months. In the patients with normal kidneys, with lupus glomerulitis and membranous lupus glomerulonephritis, on the other hand, the disease did not progress in most instances despite the fact that only small doses of prednisone were used to treat the majority of these patients.

To the physician caring for the patient with SLE, therefore, it is important to distinguish on clinical grounds and through use of laboratory tests the nature of the underlying renal involvement. This paper examines a number of clinical and laboratory findings in relation to the histology of the renal biopsies in 87 patients with SLE. These correlative studies were made in the hope that they might yield information which could lead the physician to make a confident

From the Renal-Nutrition Unit, Division of Medicine, Presbyterian-St. Luke's Hospital. Department of Medicine, Research and Educational Hospitals, and the Departments of Medicine and Pathology, University of Illinois College of Medicine

¹ Assistant Attending Physician, Presbyterian-St. Luke's Hospital; Research Associate Professor of Medicine, University of Illinois College of Medicine. Established Investigator of The American Heart Association, supported by the Chicago and Illinois Heart Associations.

² Present address Department of Metabolism, Division of Medicine, Walter Reed Army Institute of Research, Washington, D.C.

³ Professor of Pathology, University of Illinois School of Medicine. Pathologist, Research and Education Hospital, Chicago.

Supported by N.I.H. grants H-2253 and A-5374

diagnosis of the type of underlying renal involvement in patients with SLE. If such a confident diagnosis of the underlying renal pathology could be made it would no longer be necessary to do renal biopsies on these patients. Unfortunately, we are unable to demonstrate that the correlation between the underlying histologic findings and any clinical parameters is yet good enough to dispense with renal biopsy in patients with SLE.

MATERIAL AND METHODS

Systemic lupus erythematosus was diagnosed by the occurrence in combination of a variety of abnormal clinical findings and laboratory tests. The presence of LE cells was not regarded as a *sine qua non* for the diagnosis of SLE, although LE cells and/or antinuclear antibodies were found in the serum of almost all patients. Patients in whom the diagnosis of SLE was considered to be equivocal, e.g. possible rheumatoid arthritis, possible SLE, are not included in this discussion. The methods of study of the patients and the methods of analysis of the histologic specimens have been described in detail elsewhere^(1,2,3). Semiquantitative histologic analyses were made on each renal biopsy and autopsy specimen and a scale from normal (0) to extremely severe (4+) was used. The overall degree of damage to glomeruli, tubules, arteries, arterioles, and interstitial tissue was also graded from 0 to 4+. Some of the histologic changes were considered to be evidence of activity of lupus nephritis^(2,3). The extent of involvement of each glomerulus with local necrosis, karyorrhexis, hematoxylin bodies, fibrinoid, hyaline thrombi, wire loops and cellular proliferation was taken into account in assessing the activity of the lesions which was graded independently on a scale from 0 to 4+. The grading of activity was based on an overall impression rather than on a summation of the degree of involvement of each individual component. When the semiquantitative evaluation had been completed one of the following four diagnoses was made:

normal, lupus glomerulitis, active lupus glomerulonephritis and membranous lupus glomerulonephritis.

Normal were those specimens in which there was no histologic evidence of renal involvement. *Lupus glomerulitis* describes a lesion characterized by mild local and focal changes in the glomerular tuft with no significant tubular or interstitial changes. *Active lupus glomerulonephritis* is a lesion in which the glomeruli were involved together with definite tubular and interstitial changes. Parts of each glomerulus were affected rather than whole glomeruli. In addition to basement membrane thickening local necrosis with obliteration of capillary loops, karyorrhexis and fibrinoid changes usually affected some but not all glomeruli. These lesions occurred in association with areas of glomerular hypercellularity. Hematoxyphil bodies were found in some instances, hyaline thrombi more rarely, and wire loop lesions, with fibrinoid on the endothelial side of the basement membrane were seen frequently. *Membranous lupus glomerulonephritis* was a lesion in which the glomeruli were involved together with definite tubular and interstitial changes but in which the only striking abnormality in the glomerulus was a diffuse thickening of the glomerular basement membrane. Lesions indicative of activity were not seen.

RESULTS

In the initial renal biopsy of the 87 patients studied the following were the findings: normal kidneys, 10 patients; lupus glomerulitis, 23 patients; membranous lupus glomerulonephritis, 7 patients; and active lupus glomerulonephritis, 47 patients. The clinical and laboratory data are compared with the histologic analyses in Figures 1 to 7 and Tables I and II.

Urinary sediment. Fresh, early morning specimens of urine were examined after fluid had been withheld overnight. The number of erythrocytes and leukocytes per high power field in the urine at the time of the initial renal biopsy is shown

TABLE I

Percentage incidence of certain clinical extrarenal manifestations of SLE in 87 patients with SLE in whom renal histologic studies were made.*

Symptoms or signs	All 87 patients	Histologic subgroups			
		Normal kidney (10 patients)	Lupus glomerulitis (23 patients)	Membranous lupus glomeru- lonephritis (7 patients)	Active lupus glomeru- lonephritis (47 patients)
	%	%	%	%	%
Arthritis and arthralgia	86.5	80	82	86	87
Skin lesions	72.5	80	61	86	75
Pleurisy and pleural effusion	64.5	70	48	71	70
Loss of hair	56.5	60	35	71	64
Enlarged liver	46.0	40	35	43	53
Enlarged lymph nodes	45.5	40	35	29	54
Enlarged spleen	26.5	10	17	29	34
Pericardial and myocardial involvement	25.5	0	17	43	32
Raynaud's phenomenon	23.0	40	13	28	24
Peritoneal involvement	20.0	10	9	14	27
Central nervous system involvement	13.0	10	26	27	4

*The data are expressed in percentages for ease of comparison. The figures must be interpreted conservatively as the numbers in the subgroups are small.

TABLE II

Percentage incidence of certain abnormal laboratory tests in 87 patients with SLE in whom renal histologic studies were made.*

Laboratory Tests	No. of patients in whom the test was done	All patients	Histologic subgroups			
			Normal kidney (10 patients)	Lupus glomerulitis (23 patients)	Membranous lupus glomeru- lonephritis (7 patients)	Active lupus glomeru- lonephritis (47 patients)
		%	%	%	%	%
Positive LE cell test	84	83.0	66	86	66	86
Positive antinuclear antibody	53	94.2	83	94	100	96
Thymol turbidity (> 4 Maclagan units)	70	84.4	100	86	100	66
Leucocyte count (< 4500/cu mm)	84	33.3	20	30	33	38
Positive Coombs test	80	17.5	11	14	33	19
Positive Wasserman or Kahn reaction	74	9.5	0	11	20	9

*The data are expressed in percentages for ease of comparison. The figures must be interpreted conservatively as the numbers in the subgroups are small.

in Figures 1 and 2. Most patients with normal kidneys had no hematuria. Red cells were found in the urine of 6 of 23 patients with lupus glomerulitis and in 39 of 47 patients with active lupus glomerulonephritis. The occurrence of large numbers of leukocytes in the urine was associated more frequently with active

lupus glomerulonephritis than with any other underlying histologic pattern. Nevertheless it is clear from the data in Figure 2 that the number of leukocytes excreted in the urine did not help to make a *clear cut* differentiation between the underlying histology in the four groups of patients.

In general, casts were found in the urine of patients with lupus glomerulonephritis but they were not in themselves a good indication of whether or not there was underlying active disease. Red cell casts were most frequently observed in patients in whom there was active

lupus glomerulonephritis. "Telescoped" urinary sediments were observed only in active lupus glomerulonephritis.

Proteinuria. The degree of proteinuria at the time of the initial renal biopsy in the 87 patients is shown in Figure 3. Urines of all 10 patients with normal

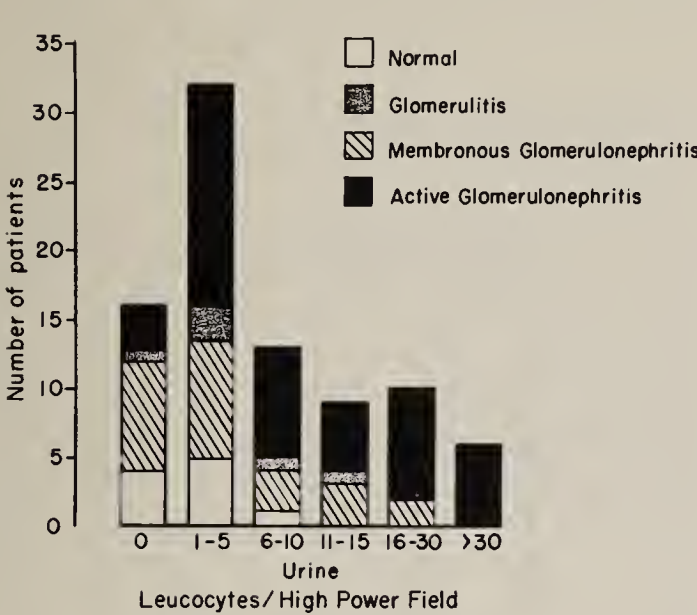


Fig. 1.—The relationship between the underlying renal histology and the excretion of red blood cells in the urine of patients with SLE.

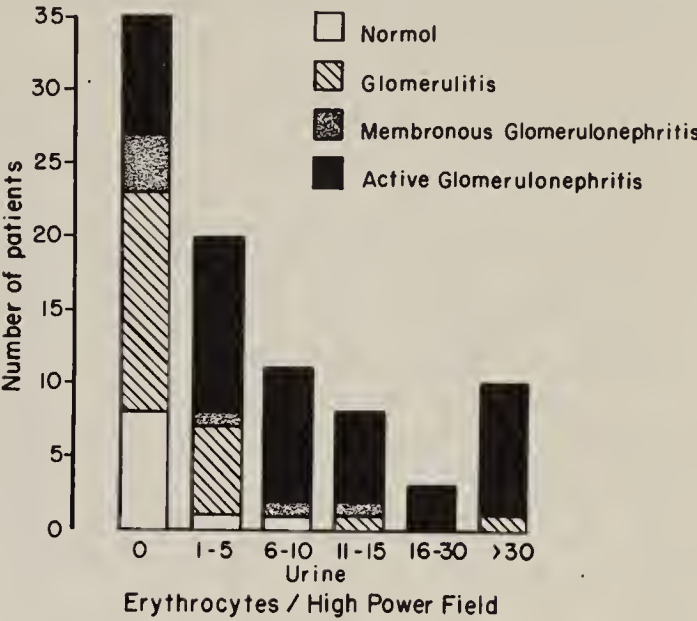


Fig. 2.—The relationship between the underlying renal histology and the excretion of white blood cells in the urine of patients with SLE.

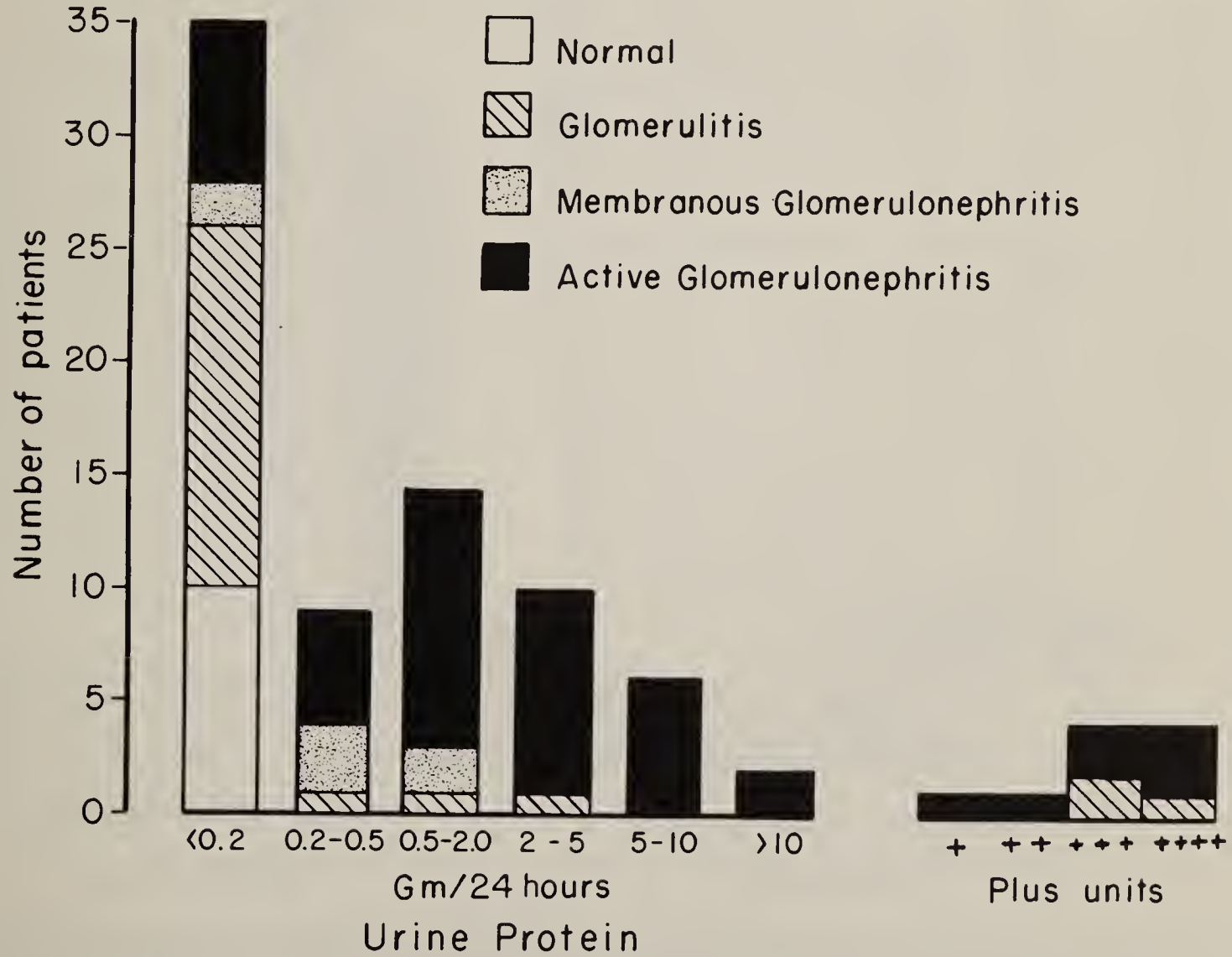


Fig. 3.—The relationship between the underlying renal histology and proteinuria in patients with SLE.

kidneys contained no protein. Sixteen of the 23 patients with lupus glomerulitis had no significant proteinuria but protein was found in the urine of the other 7. Proteinuria was found in most patients with membranous glomerulonephritis and active lupus glomerulonephritis. Nevertheless 9 patients with these under-

lying diagnoses had no significant proteinuria.

When the detailed analysis of all 176 renal biopsy and autopsy specimens was examined in relation to the urine protein excretion it was found that the magnitude of the proteinuria correlated well with overall histologic severity of the lesions,

LUPUS NEPHRITIS

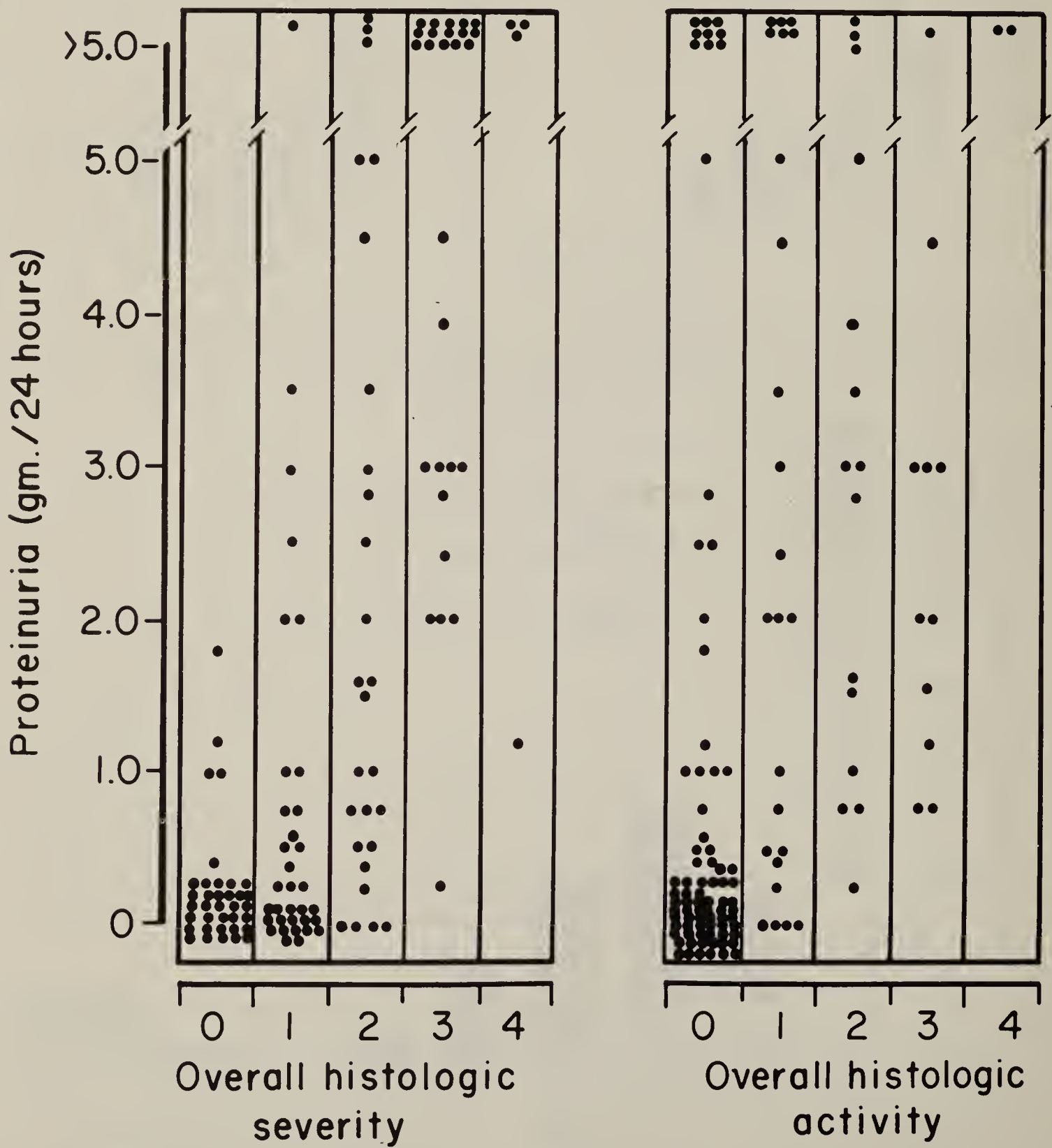


Fig. 4.—The relationship between proteinuria and the underlying histologic severity and activity of the renal lesions of patients with SLE. Note that the degree of proteinuria reflects the underlying histologic severity but is not related to the underlying histologic activity.

with the degree of glomerular basement membrane thickening and the degree of tubular degeneration, but not with the degree of histologic activity of the lesions (Figure 4). Nephrotic syndrome occurred in one of seven patients with membranous lupus glomerulonephritis, and in 30 of 47 patients with active lupus glomerulonephritis.

In the urines of 69 of the 87 patients there was proteinuria (>200 mg/24 hours) and/or hematuria (>2 rbc/hpf) and/or pyuria (>2 wbc/hpf). By these criteria urinary abnormalities were found in 6 of the 10 patients with normal kidneys, in 12 of the 23 patients diagnosed as lupus glomerulitis, in 6 of the 7 with membranous lupus glomerulonephritis, and in 45 of 47 with active lupus glomerulonephritis.

Serum creatinine and urea nitrogen levels. The serum creatinine and urea nitrogen levels (Figure 5) were within normal limits at the time of the initial biopsy in almost all patients with normal kidneys, lupus glomerulitis and membranous lupus glomerulonephritis. In patients with active lupus glomerulonephritis the average serum creatinine and urea nitrogen levels were increased but the values observed in approximately one-half of the patients with active lupus glomerulonephritis were within normal limits. In Figure 6 the creatinine clearance and urea clearance tests are plotted against the degree of severity of the renal histologic findings whatever the underlying histologic diagnosis. The data are plotted for clearances done at the time of the initial and all subsequent renal biopsies.

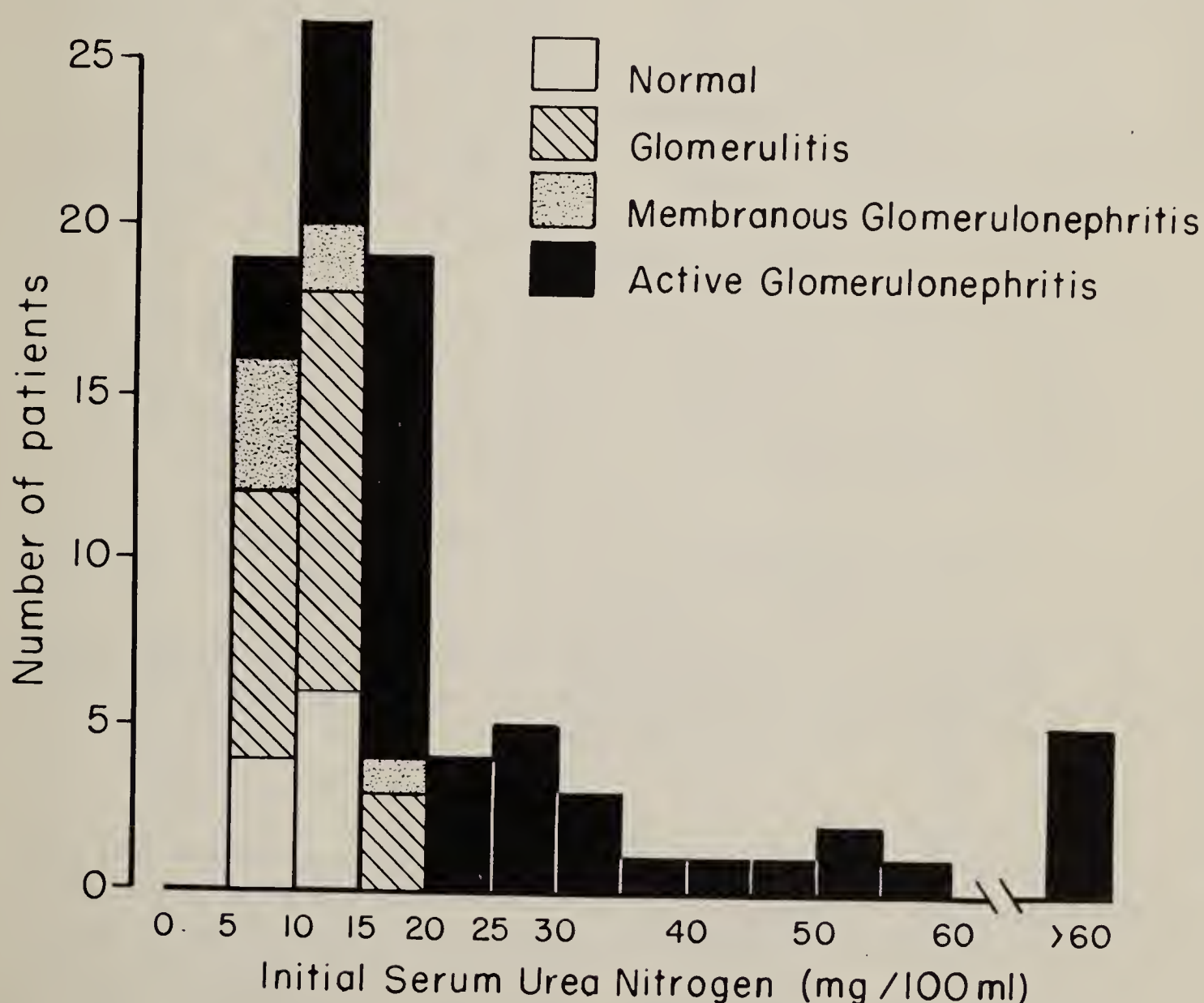


Fig. 5.—The serum urea nitrogen levels at the time of the initial renal biopsy in 87 patients with SLE. Note that the initial serum urea nitrogen level was within normal limits in many patients with active lupus glomerulonephritis.

LUPUS NEPHRITIS

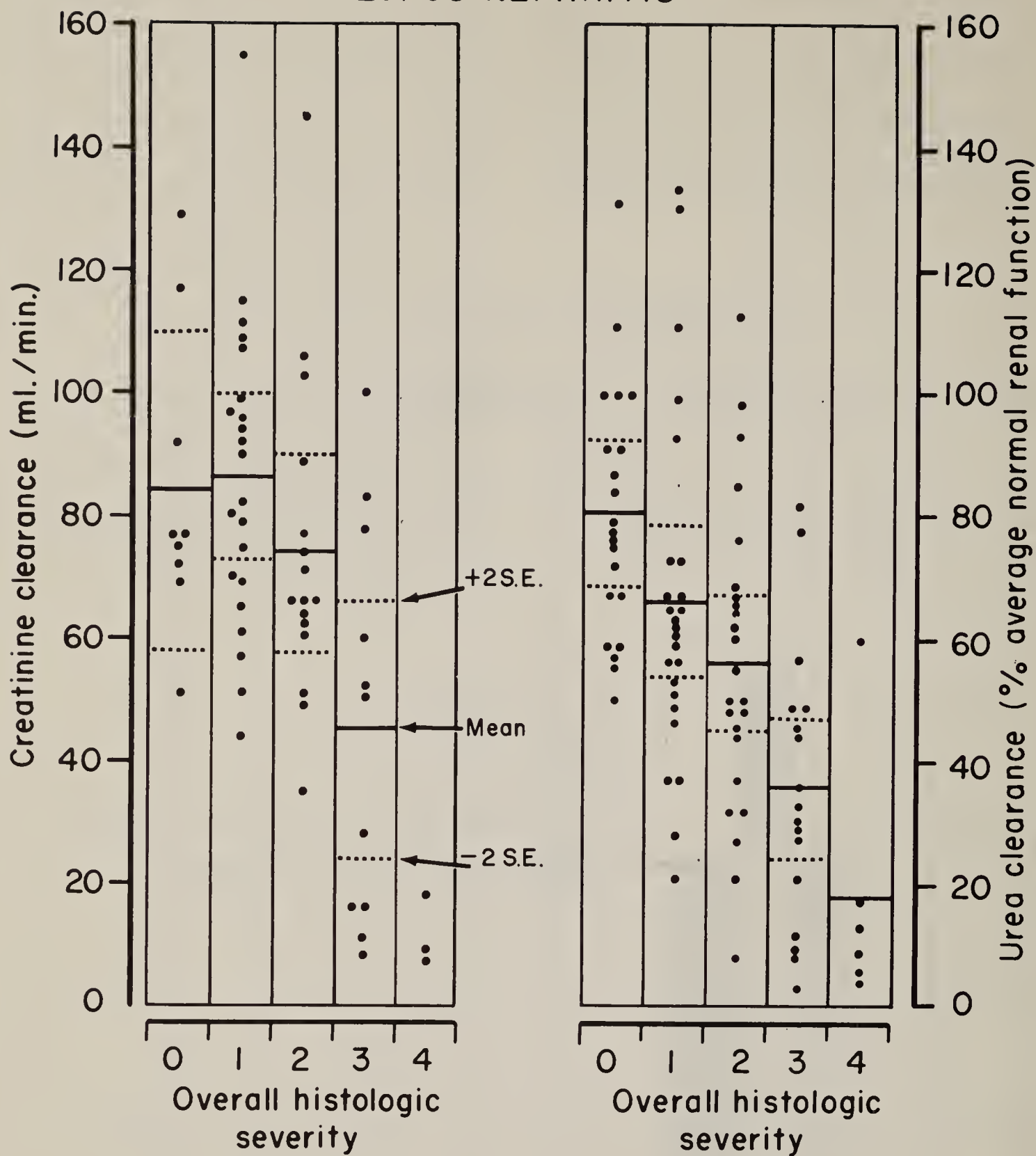


Fig. 6.—Creatinine and urea clearances in patients with SLE. There is an inverse relationship between the creatinine and urea clearances and the underlying histologic severity. As expected, however, the overlap at each degree of severity is considerable.

Although there was the expected inverse relationship between the histologic severity of the renal lesions and creatinine and urea clearances, the overlap was considerable; in many patients, the severity of whose kidney lesions was graded 2+, the creatinine and urea clearances were within the normal range.

Although the creatinine and urea clearances reflected overall histologic severity, they did not correlate with the degree of histologic activity of the renal lesions. However, most patients in whom the disease was active had decreased urea and creatinine clearances. The correlation of the urea clearance with the sever-

ity of glomerular damage was good as has been indicated. Equally good correlations were obtained with the severity of interstitial damage or of tubular damage, probably related to the observation that tubular and interstitial damage follow on and reflect the degree of glomerular damage in the kidneys of patients with SLE.

The amount of phenosulfonphthalein (PSP) excreted in the 15 minutes after an intravenous injection of 6 mg was also examined. The PSP excretion also reflected the severity of histologic damage to the glomeruli and tubules, but not the degree of activity of the lesions. As with the other tests there was considerable overlap between the degrees of histologic abnormality.

Clinical symptoms and signs. The level of the systolic and diastolic blood pressure (Figure 7) was examined in relation to the histology of all the renal biopsies. The average blood pressure was higher in the presence than in the absence of *arterial* damage, but did not increase with increasing degrees of arterial abnormal-

ity. The average blood pressure increased significantly as the glomerular and *arteriolar* damage became more severe. Thus the blood pressure was a good reflection of the degree of underlying glomerular and arteriolar damage, despite the fact that many other factors may have influenced the blood pressure levels in these patients. For example the varying and sometimes large doses of prednisone used to treat these patients might have been expected to influence the blood pressure.

The incidence of individual clinical symptoms and signs is shown in Table I. The observations in this table relate the clinical abnormalities found before or at the time of the first renal biopsy to the renal histology. In the 87 patients, considered as a whole, the incidence of the various extrarenal manifestations of SLE was comparable to that described by other authors.^{7,8,9,10} Although the numbers are small there was no significant difference between the four histologic groups in the incidence of the clinical extrarenal manifestations of SLE. The

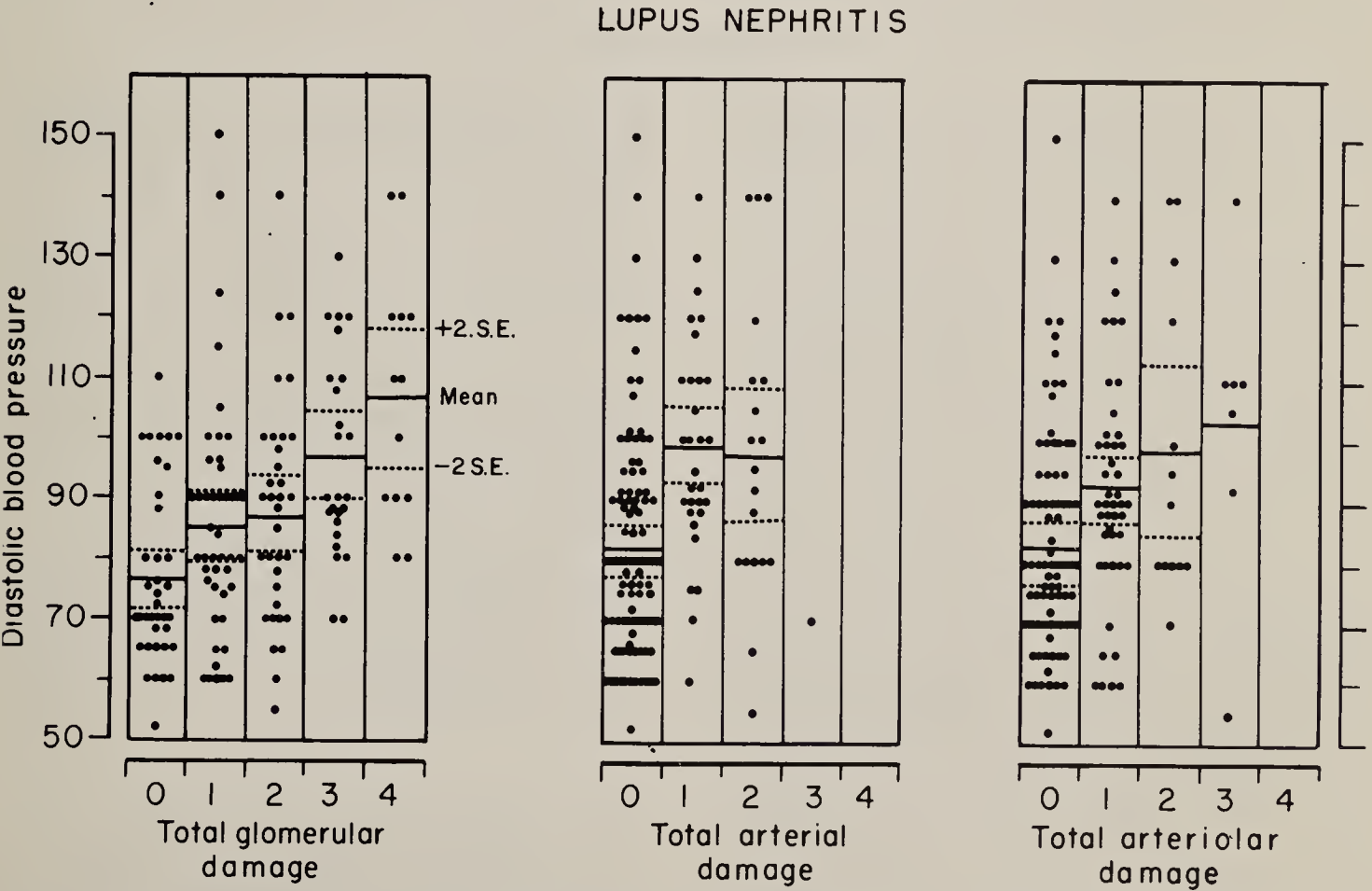


Fig. 7.—The relationship between the underlying renal histology and diastolic blood pressure in patients with SLE. Note the step-wise increase in diastolic blood pressure with increasing severity of glomerular and arteriolar damage.

incidence of abnormal findings in certain laboratory tests is shown in Table II. No significant difference between the four histologic groups was found in the incidence of positive results in any of these tests.

Thus the extrarenal clinical and laboratory findings did not help to distinguish active lupus glomerulonephritis from the other three histologic groups.

DISCUSSION

From the clinical point of view active lupus glomerulonephritis can usually be diagnosed with confidence in most patients with SLE in whom proteinuria, hematuria, pyuria, cylinduria and azotemia are found. In active lupus glomerulonephritis with moderate or severe azotemia the prognosis is usually grave, and treatment with small or large doses of cortisol and its analogs, nitrogen mustard, 6-mercaptopurine etc. has done little to alter the picture.

On the other hand, many patients with active lupus glomerulonephritis have no azotemia (Figure 5). The disease is nevertheless rapidly progressive, but at this early stage may be halted in many patients by the prolonged use of large doses of prednisone.^{2,3} The interrelationships between clinical findings and renal histology set forth in this paper indicate that the distinction between active lupus glomerulonephritis, lupus glomerulitis and membranous lupus glomerulonephritis cannot always be made with certainty on the basis of clinical evaluation, urinalysis and simple tests of renal function. Thus in the present state of our knowledge renal biopsy is still the most reliable guide to the diagnosis, prognosis and therapy of renal involvement in SLE.

It is clear that more sophisticated tests are necessary in the study of patients with SLE. Stevens and Knowles¹¹ have suggested that the absolute and/or relative amounts of gamma globulin were increased in the urine of patients with "active" lupus nephritis, but this has not yet been confirmed by others, including ourselves.¹² The finding of relatively large amounts of gamma globulin in urine is certainly not specific for lupus nephritis, and it occurs frequently in pyelonephritis.¹³ Immunoelectrophoretic analyses of urine proteins in patients with SLE indicate that many serum proteins are excreted, and that the pattern of proteins excreted varies from patient to patient.¹² Townes, Stewart and Osler have shown that the occurrence in combination of high levels of nucleoprotein reactive gamma globulin and low levels of serum complement was frequent in active lupus nephritis,¹⁴ and Seligmann found low levels of β_1A globulin—a component of complement—in active lupus nephritis.¹⁵ However, none of these tests, either severally or in combination, can yet distinguish with certainty active SLE without significant renal involvement from active SLE with active lupus glomerulonephritis.

ACKNOWLEDGEMENTS

We wish to thank Miss Joanna Puniska and Mr. Roy McClain for their able technical assistance.



REFERENCES

1. Muehrcke, R. C., Kark, R. M., Pirani, C. L. and Pollak, V. E.: Lupus nephritis: A clinical and pathologic study based on renal biopsy. *Medicine (Baltimore)* 36:1, 1957.
2. Pollak, V. E., Pirani, C. L. and Schwartz, F. D.: The natural history of the renal manifestations of systemic lupus erythematosus. *J. Lab. Clin. Med.* 63: 1964 (in press).
3. Pollak, V. E., Pirani, C. L. and Kark, R. M.: Effect of large doses of prednisone on the renal lesions and life span of patients with lupus glomerulonephritis. *J. Lab. Clin. Med.* 57:495, 1961.
4. Holman, H.: Systemic Lupus Erythematosus. A review of certain recent developments in the study of this disease. *J. Pediat.* 56:109, 1960.
5. MacKay, I. R. and Taft, L. I.: Renal biopsy with particular reference to the study of diabetes mellitus, systemic lupus erythematosus and subacute glomerulonephritis. *Australasian Ann. Med.* 10:178, 1961.
6. Wilson, R. M., Maher, J. F. and Schreiner, G. E.: Lupus nephritis; clinical and histologic survey. *Arch. Int. Med.* 111:429, 1963.
7. Harvey, A. M., Shulman, L. E., Tumulty, P. A., Conley, C. L. and Schoenrich, E. H.: Systemic lupus erythematosus: Review of the literature and clinical analysis of 138 cases. *Medicine (Baltimore)* 33:291, 1954.
8. Dubois, E. L.: The effect of the L. E. cell test on the clinical picture of systemic lupus erythematosus. *Ann. Int. Med.* 39:1265, 1953.
9. Jessar, R. A., Lamont-Havers, W. and Ragan, C.: Natural history of lupus erythematosus disseminatus. *Ann. Int. Med.* 38:717, 1953.
10. Shearn, M. A. and Pirofsky, B.: Disseminated lupus erythematosus. *Arch. Int. Med.* 90:790, 1952.
11. Stevens, M. B. and Knowles, B.: Significance of urinary gamma globulin in lupus nephritis. I. Electrophoretic analysis. *New Engl. J. Med.* 267:1159, 1962.
12. Barcelo, R., Miyasato, F. and Pollak, V. E.: Unpublished observations.
13. Tidstrom, B.: Urinary protein in glomerulonephritis and pyelonephritis. Electrophoretic analyses. *Acta. Med. Scandinav.* 174:385, 1963.
14. Townes, A. S., Stewart, C. R., Jr. and Osler, A. G.: Immunologic studies of systemic lupus erythematosus. II. Variation of nucleoprotein-reactive gamma globulin and hemolytic serum complement levels with disease activity. *Bull. Johns Hopk. Hosp.* 112:202, 1963.
15. Seligmann, M.: Examens biologiques permettant de rattacher une néphropathie au lupus érythémateux disséminé. *Actualités Néphrologiques de L'Hôpital Necker, Paris, Flammarion*, 1961.

CONTRAST ARTHROGRAPHY OF THE KNEE JOINT

RICHARD E. BUENGER, M.D.¹

Arthrography is well established as a method of outlining the internal structures of the joints. It is particularly valuable in those joints having an articular disc (temporo-mandibular and knee) and joints with liberal capsular spaces and bursal communications, (shoulder, hip, knee). The knee joint has been the most frequently studied because of the relatively common occurrence of surgically correctable traumatic, inflammatory and degenerative diseases of this joint.

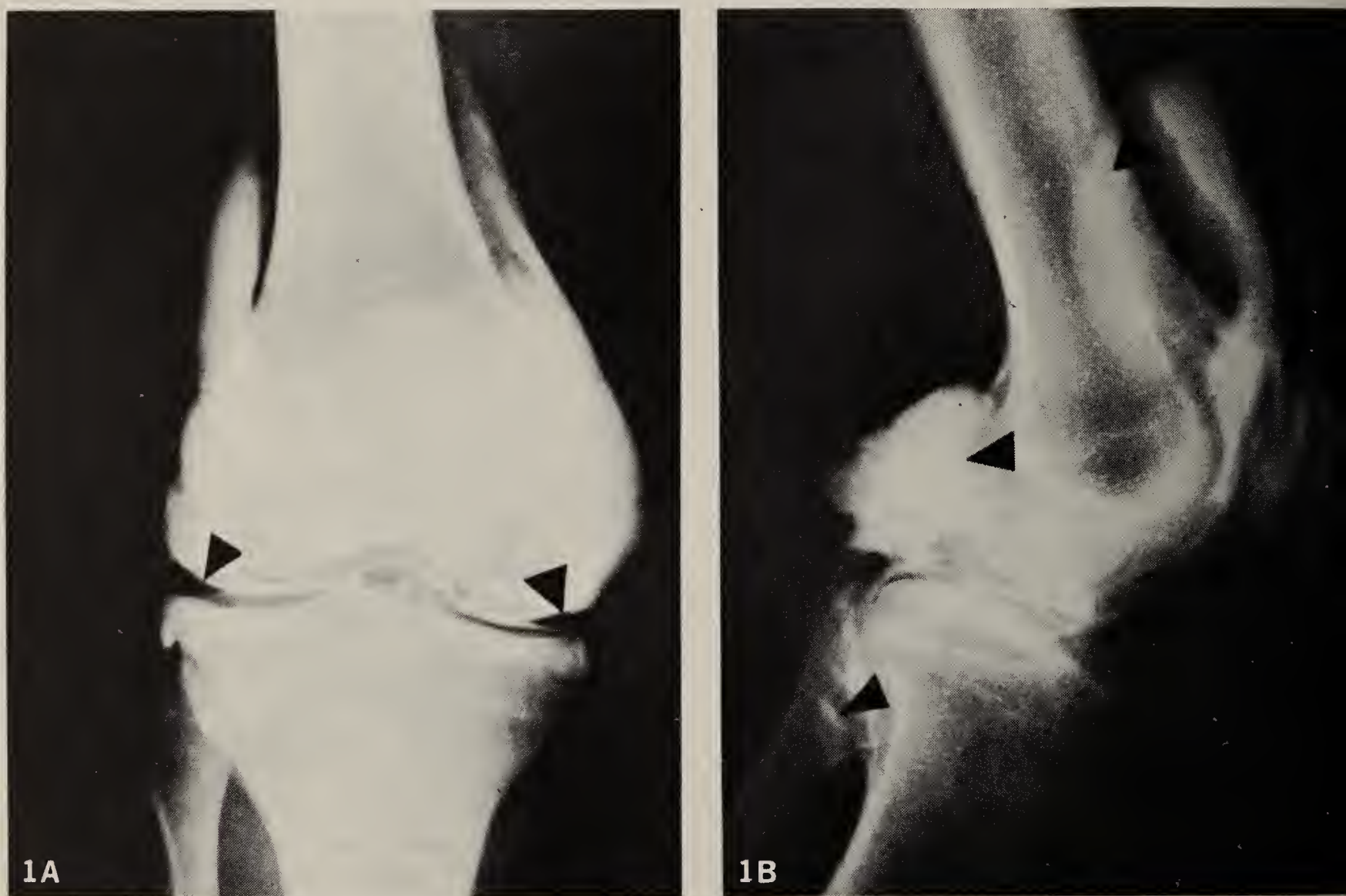


Fig. 1.—Normal Arthrogram: A. Antero-posterior view.—Arrows indicate triangular-shaped menisci B. Lateral view.—Upper arrow indicates suprapatellar bursa. Middle arrow indicates the semimembranosus-gastrocnemius bursa "Baker's Cyst" posteriorly. Lower arrow points to bursa along popliteus tendon.

From the Division of Radiology and Nuclear Medicine

¹ Attending Radiologist, Presbyterian-St. Luke's Hospital; Associate Professor, University of Illinois College of Medicine

Arthrography is a quick, safe, painless procedure which may be performed at the time of aspiration of synovial fluid for culture or cytologic examination. Both gas and iodinated agents have been

used for contrast. We prefer the radio-paque liquid which permeates into tears and bursal sacs more quickly and completely. (Fig. 1).

Most of the emphasis of arthrography has been in the diagnosis of meniscus lacerations in patients with an atypical history or vague physical findings. (Fig. 2) This is a very accurate examination if care is taken to avoid over-interpretation in those patients with suboptimal roentgenograms.

Arthrography of the knee has been helpful and should be used more frequently in some common, non-traumatic clinical problems such as:

1. Recurrent effusion. (Fig. 3)
2. Para-articular swelling or tenderness. (Fig. 4)
3. Grinding sensation or locking not explained with routine roentgenograms. (Fig. 5)
4. Abnormal calcifications noted on plain roentgenograms. (Fig. 6)
5. Synovitis of questionable diagnosis, or prior to therapy. (Fig. 7)

It is hoped that these illustrated examples will serve as a stimulus to the more frequent use of the valuable diagnostic procedure of contrast arthrography of the knee.

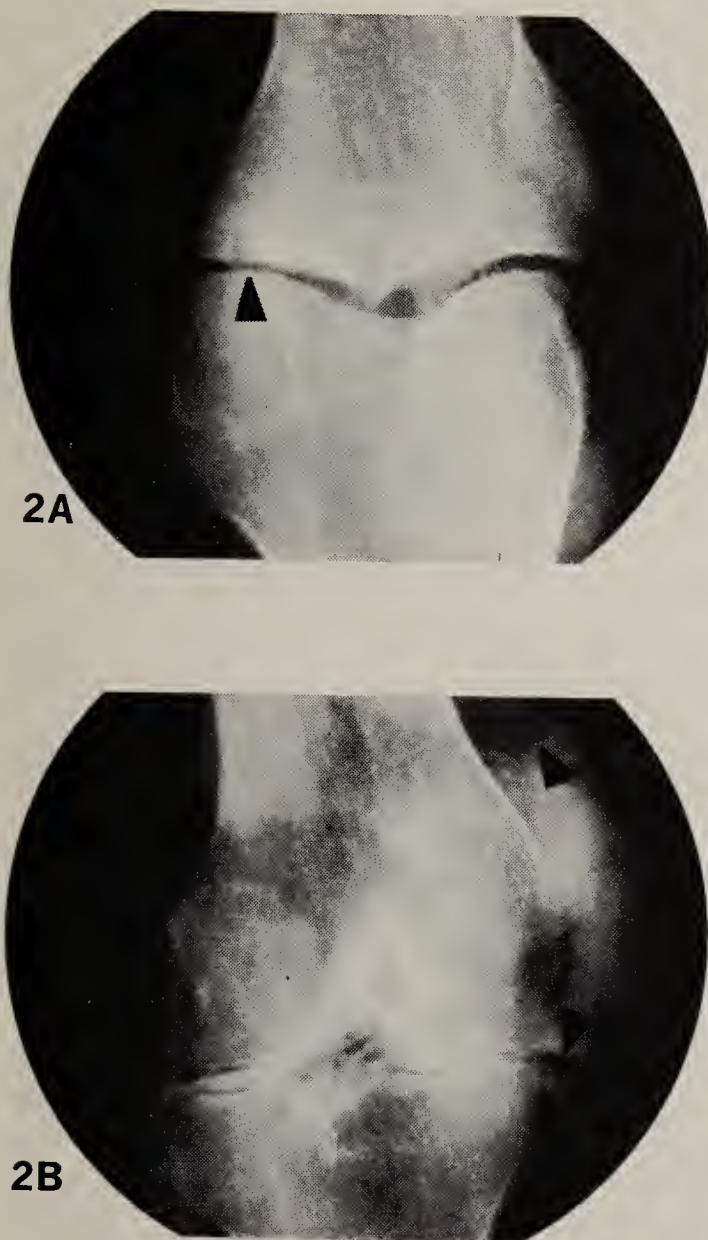


Fig. 2.—Post-Traumatic Arthritis and Synovitis: A. Plain roentgenogram shows narrowing of medial joint space (arrow) with eburnation and "cystic" lucencies at articular surface. B. Arthrogram with upper arrow indicating nodular synovial thickening and lower arrow indicating subluxation of medial meniscus away from joint—(compare with Fig. 1A). Other views demonstrated multiple tears in menisci.



Fig. 3.—Proliferative Synovitis: Arthrogram with arrows outlining localized nodular synovitis (excised surgically) in 11 year old girl with history of recurrent swelling and tenderness of knee.



Fig. 4.—Rheumatoid Arthritis: A. Plain lateral view—Arrows indicate soft tissue mass (fluid in posterior bursa). B. Opaque contrast arthrogram—Arrows indicate papillary synovial excrescences in posterior bursa—Note smooth synovia in joint and suprapatellar pouch.

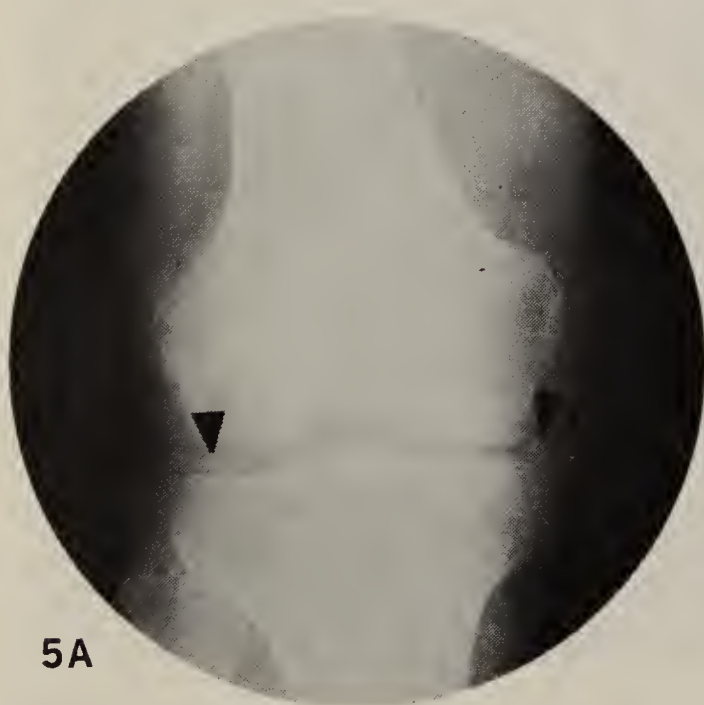


Fig. 5.—Calcified Menisci: A. Plain roentgenogram with stippled calcifications in region of both menisci. (arrows). B. Contrast arthrogram shows normal menisci containing calcium.

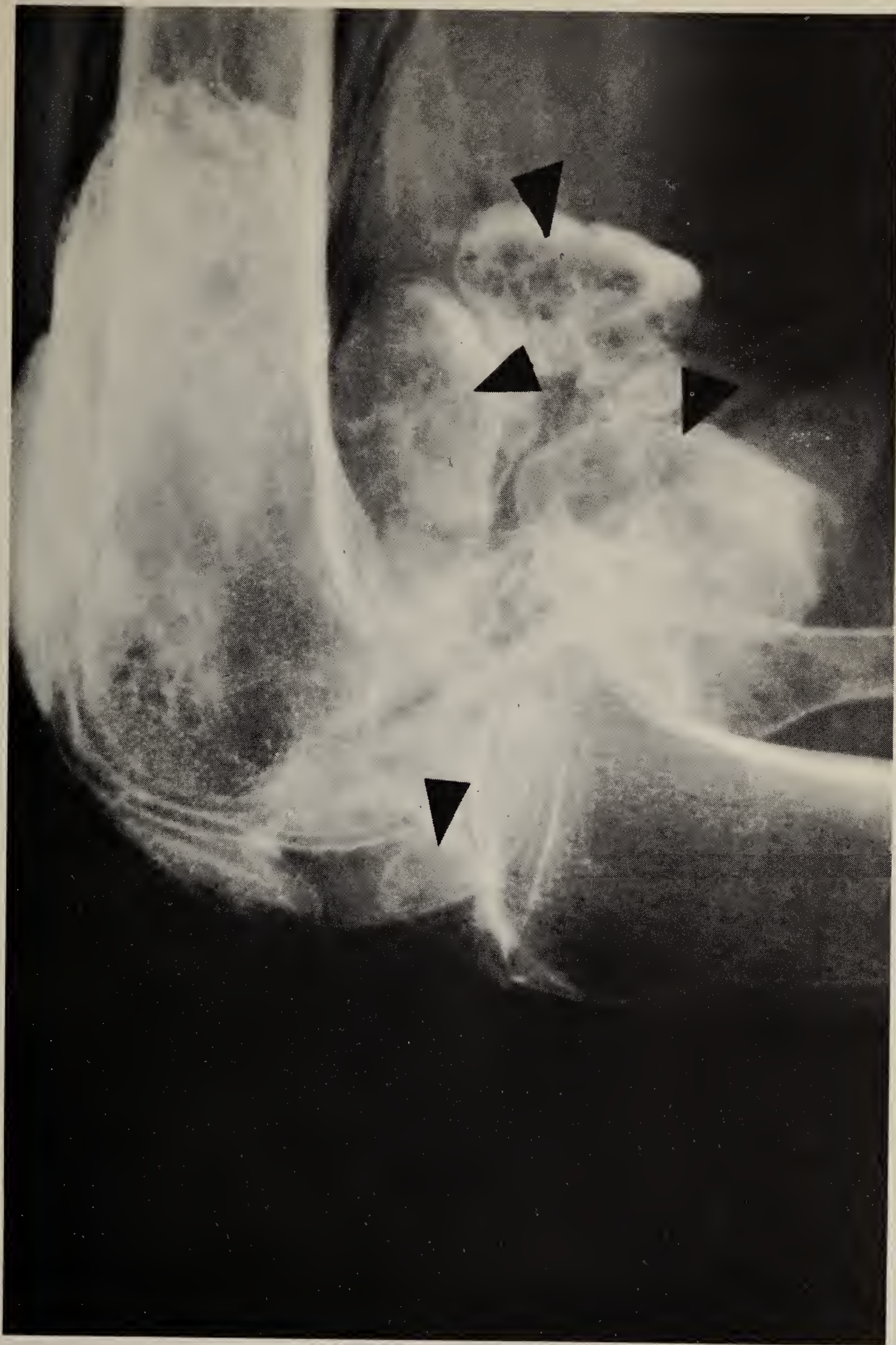


Fig. 6.—Chondromatosis: Opaque contrast arthrogram (following inconclusive gas arthrogram) showing many communicating posterior pouches containing loose chondromatous material (arrows) which were subsequently excised. Chondromata also present anteriorly in joint. (arrow).



Fig. 7.—Villonodular Synovitis: Lateral view of arthrogram—Note filling of large posterior pouch. Arrows indicate areas of villonodular synovitis in the suprapatellar and posterior pouches.

REFERENCES

1. Bonnin, J. G. and Boldero, J. L.: Air Arthrography of the Knee Joint. *Surg. Gynec. & Obst.*, 85: 64-70, 1947.
2. Lindblom, K.: Arthrography of the Knee. A Roentgenographic and Anatomic Study. *Acta Radiol. Supplement No. 74*, 1948.
3. Norgaard, F.: Arthrography of the Mandibular Joint. *Acta Radio.*, 25:679-685, 1944.
4. Samilson, R. L., Raphael, R. L., Post, L., Noonan, C., Siris, E. and Raney, F. L., Jr.: Shoulder Arthrography. *J.A.M.A.*, 175:773-338, 1961.

THE TEMPORO-MANDIBULAR JOINTS IN RHEUMATOID ARTHRITIS

MAURICE L. BOGDONOFF, M.D.¹

X-ray examination of the temporo-mandibular joints can be helpful in diagnosing rheumatoid arthritis. None of the morphologic features of arthritis at the temporo-mandibular joint is diagnostic of rheumatoid arthritis. This disease may involve the temporo-mandibular joints at any time in its course.

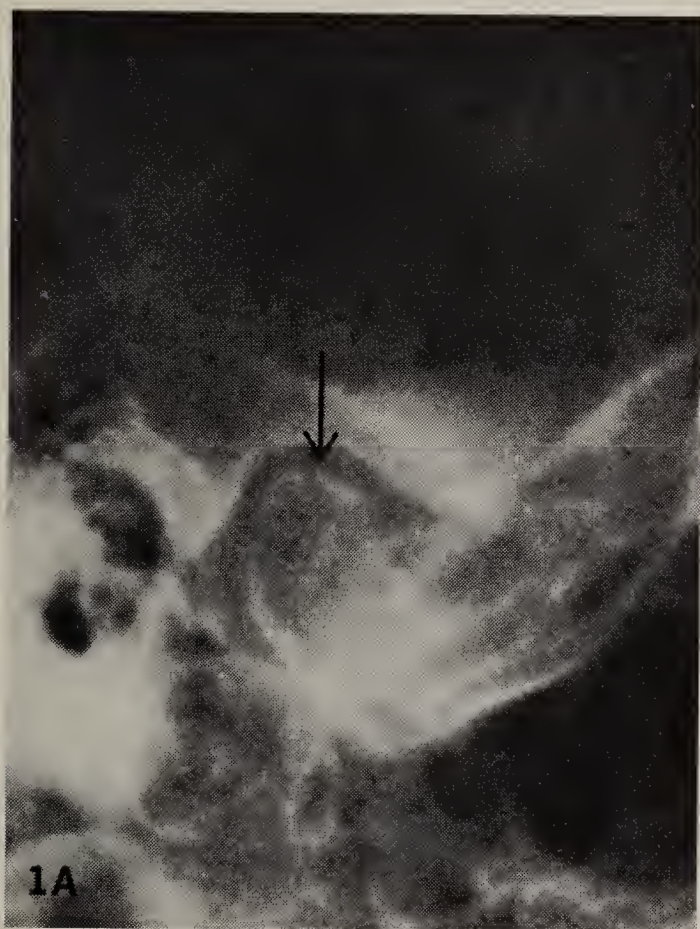


Fig. 1A and 1B.—Normal temporo-mandibular joint showing range of motion with mouth closed (1A) and open (1B).

There are documented instances of this joint being involved in the juvenile form of the disease and in occasional cases of the so-called “atypical” forms of rheumatoid arthritis.¹ The diagnosis

From the Division of Radiology & Nuclear Medicine

¹ Assistant Attending Radiologist, Presbyterian-St. Luke's Hospital; Assistant Professor, University of Illinois College of Medicine

is suggested when a bilateral, progressive arthritis has been documented. The response of the bone and joint tissues in the temporo-mandibular joint to the causative factors of rheumatoid arthritis is no different from that of other small joints. The earliest findings consist of a rather localized soft tissue swelling followed by trabecular thinning. Later, bony surface erosions appear followed by periosteal elevation secondary to proliferating scar

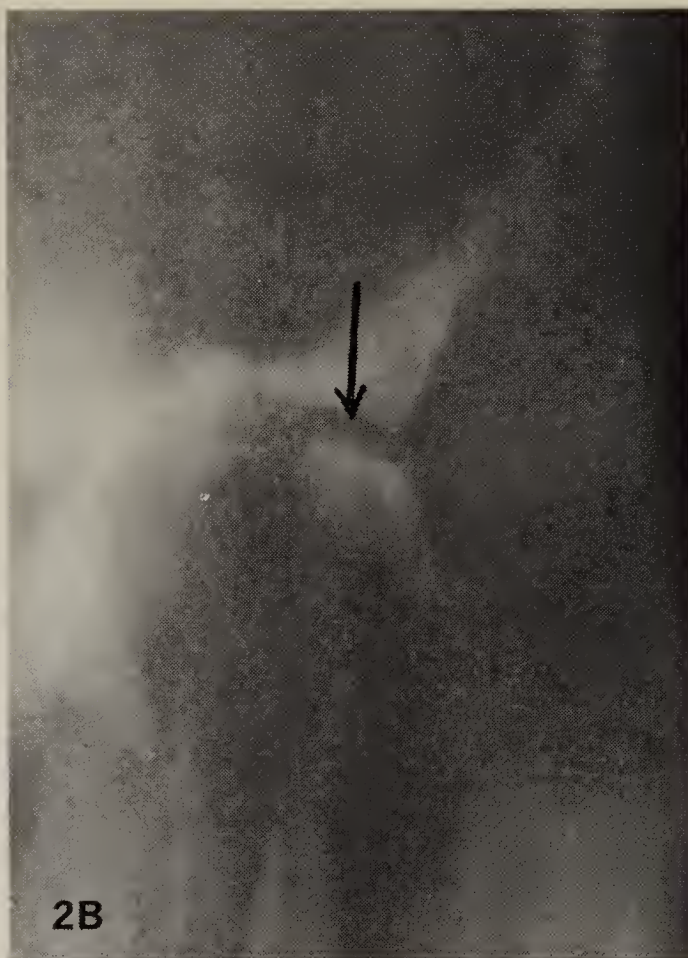
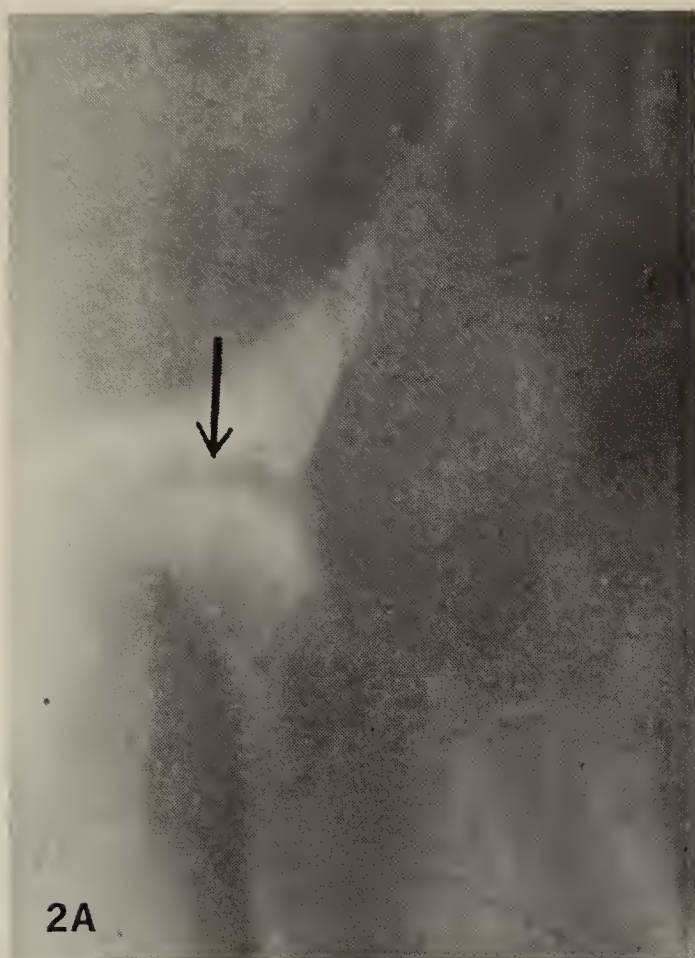


Fig. 2A and 2B.—Restricted motion and bone irregularity shown on laminographs. Jaw closed (2A) and open (2B).

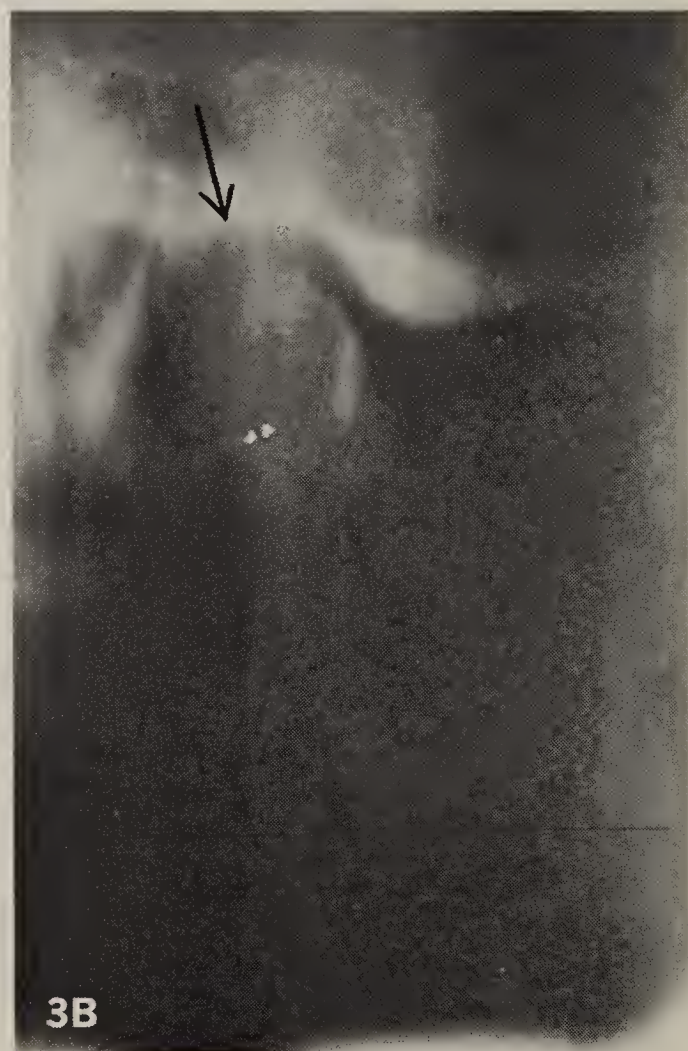
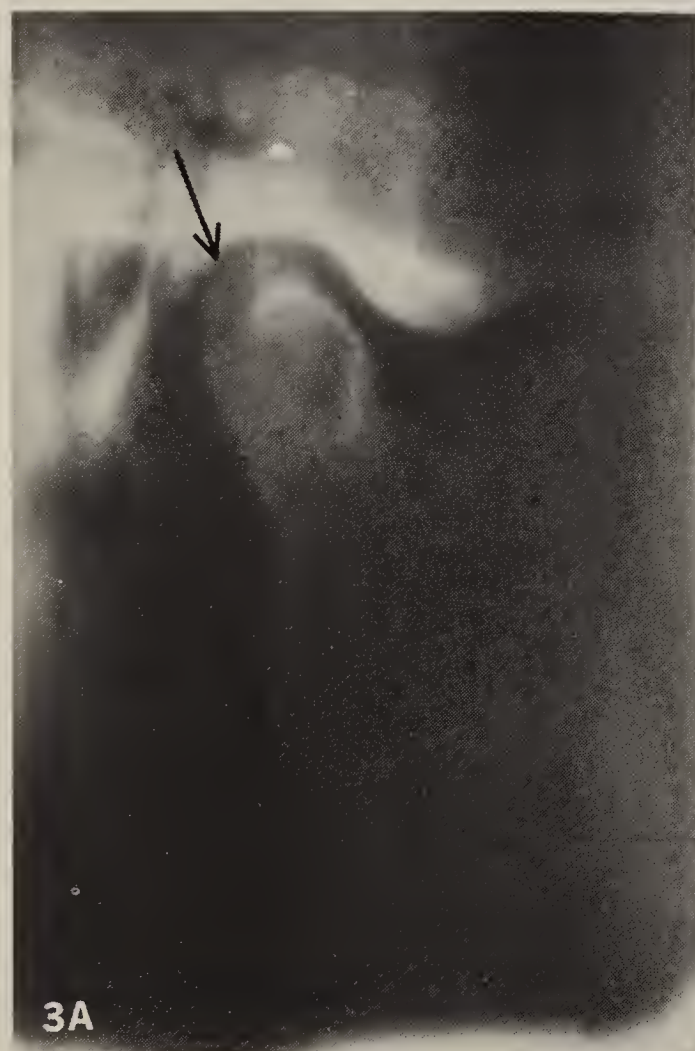


Fig. 3A and 3B.—Laminographs showing posterior thinning of condyle. This is an early change of rheumatoid arthritis.

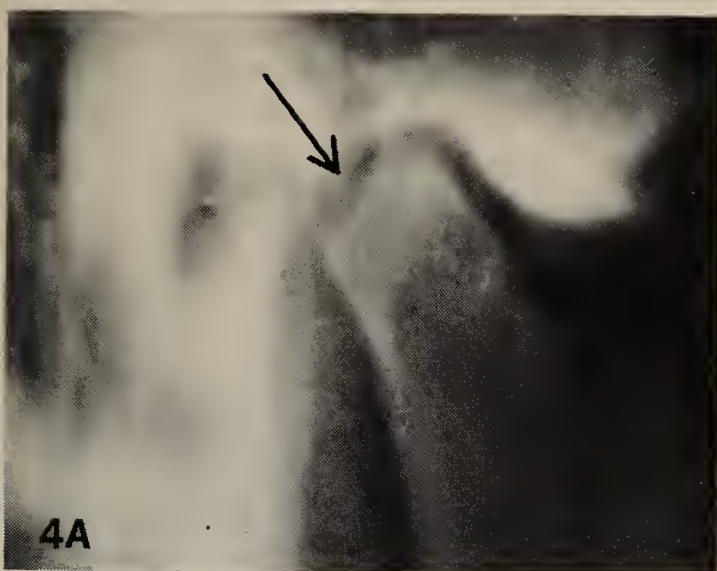


Fig. 4A and 4B.—Laminographs showing flattening of posterior portion of condyle. Note rather sharp angle of posterior mandible at junction of condyle and neck.

tissue. Irregular areas of ossification may appear at the edge of the progressing scar. Narrowing of the joint space becomes apparent later in the course and ankylosis may be demonstrated at the temporo-mandibular joint in a few patients. Films made at the time of onset of symptoms referable to the temporo-mandibular joint are usually normal.

Changes of degenerative arthritis or osteoarthritis occur in the temporo-mandibular joint. These can be confusing. Loss of bone substance, joint narrowing and the so-called productive bone change or osteophyte formation do occur in the temporo-mandibular joint, sometimes in association with dental problems such as malocclusion. They may also occur fol-

lowing trauma. Usually there is less trabecular resorption and demineralization in this degenerative group of diseases than there is in rheumatoid arthritis. There are many patients who have productive bone pathology associated with more typical pathology of rheumatoid arthritis.²

The best radiological technique for examining the temporo-mandibular joints is laminography. Films are obtained with the mouth open and closed. Approximately 15 percent of patients having rheumatoid arthritis have symptoms of the disease in the temporo-mandibular joints.³ This is usually bilateral as in other joints in this disease.

SUMMARY

Temporo-mandibular arthritis occurs in approximately 15 percent of patients having rheumatoid arthritis. It appears at variable times in the course of the disease. Radiographs made at the time of onset of symptoms are almost always normal. After the disease has persisted for a matter of months or more, radio-

logic findings compatible with rheumatoid arthritis, but not diagnostic of this disease, may be demonstrated in a majority of patients. The bone pathology of rheumatoid arthritis involving the temporo-mandibular joint is best demonstrated by laminography.

REFERENCES

1. Short, C. L., Bauer, W., and Reynolds, W. E.: *Rheumatoid Arthritis*. Cambridge, Massachusetts, Harvard University Press, 1957.
2. Thoma, K. H. and Goldman, H. M.: *Oral Pathology*, St. Louis, Mo., C. V. Mosby, 1960, p. 851-865.
3. Lewis-Fanning, E.: Report on an Enquiry Into the Aetiological Factors Associated with Rheumatoid Arthritis, *Ann. Rheumat. Dis.* 9: Supplement 94 pages, 1950.

ADVERSE EFFECTS OF DRUG THERAPY IN RHEUMATOID ARTHRITIS

GEORGE W. STUPPY, M.D.¹

Anti-inflammatory agents of demonstrated therapeutic value in the treatment of rheumatoid arthritis include the salicylates, gold salts, adrenal corticosteroids and corticotropin, phenylbutazone-antipyrine and anti-malarial 4-aminoquinoline compounds. All these drugs produce some beneficial clinical effects of varying degree in the active stages of rheumatoid arthritis but they frequently cause undesirable and serious reactions, and they should be prescribed only with full knowledge of their potential dangers, as well as their possible untoward actions in patients with other intercurrent disease. Some of the general pharmacologic actions of these drugs are fairly well recognized but their basic mechanisms in the therapy of rheumatoid arthritis are obscure. Experimentally it has been shown that they exert an inhibitory influence on one or more metabolic processes at an enzyme level, the most consistent of which may be the inhibition of mucopolysaccharide synthesis by connective tissue.^{1,2,8,9,17,25}

Salicylates: Large doses of salicylates can produce "salicylism" with symptoms of headache, dizziness, tinnitus and decreased hearing, sweating and fever, nausea and vomiting with abdominal discomfort. Salicylate poisoning is manifested by occult gastro-intestinal bleeding, occasional gastro-intestinal hemorrhage, mental confusion, delirium and coma. In 1952 113 deaths due to salicylate poisoning were reported in the United States; 86 were children under 5 years of age. In England and Wales 200 deaths are reported annually, consisting of pre-school children and young adults 15 to 24 years of age and a larger group of elderly patients.^{1,2,3,4}

The effective analgesic level of salicylate in adults is 25 to 30 mgm./100 ml. of serum and the margin between toxic and therapeutic levels is about 10 mgm./100 ml. 50 to 80 percent of the salicylate ion is normally bound to plasma albumin, and in those rheumatoid arthritis patients with hypoalbuminemia plasma levels are consequently lower. Almost all of ingested salicylate is excreted in the urine in 48 hours, depending upon the rate of glomerular filtration and proximal tubular secretion, urine flow and pH. About 85 percent is excreted as free salicylate in alkaline urine while in acid urine only 10 percent may be excreted.^{1,2}

Acid-base equilibrium is altered in different ways, apparently depending upon the age of the patient, to some extent. Fixed acids are increased and plasma bicarbonate is reduced. Metabolism is increased with increased temperature and carbon dioxide production. A plasma concentration of 12 to 16

From the Division of Medicine

¹ Attending Physician, Presbyterian-St. Luke's Hospital; Clinical Professor of Medicine (Rush) University of Illinois College of Medicine

mgm./100 ml. may increase the sensitivity of the respiratory center 3 or 4 fold to cause a striking increase in minute respiratory volume, fall in arterial CO_2 and rise in pH. Individual sensitivity varies greatly and infants usually develop intense ketosis and acidosis; older children and adults are more likely to show respiratory alkalosis. These effects are not due to salicylate directly but are probably the result of its interference with the oxidative enzyme systems.^{5,6}

Salicylate expands plasma volume and increases intracellular body water. The O_2 uptake and O_2 consumption of tissues is increased with increased heat production and hypoglycemia, all of which cause an increase in cardiac output and work of the left ventricle, which can be detrimental to patients with cardiac disease. Glycogen is decreased in the liver and terminally in muscle and myocardium. In fatal cases fatty infiltration of the liver and kidney, petechial hemorrhages in viscera and the central nervous system, nerve cell damage and toxic encephalopathy are usually seen.¹

Occult gastro-intestinal bleeding and acute gastric hemorrhage occur frequently during salicylate administration, but the role of salicylate in the production of peptic ulceration and gastro-intestinal hemorrhage has not been clearly established, although the implications of a recent study in England are suggestive. Of 106 patients who had been hospitalized with acute gastro-intestinal hemorrhage 57 had taken aspirin 48 hours previous to onset of hemorrhage, while in a control series of 106 patients who had not taken aspirin 17 patients were admitted with gastro-intestinal hemorrhage. Another group of 38 patients who had taken 3 to 6 Grams of aspirin daily 3 to 6 days previously were found to have a fecal blood loss of 3.3 ml. daily. Fecal bleeding stopped within a few days after aspirin was discontinued. The depression of prothrombin levels observed in patients on aspirin hardly seems adequate to account for blood loss and hemorrhage. There has been no appreci-

able difference shown in plasma levels, analgesic action, clinical results or complications with the use of buffered, non-buffered or enteric coated preparations.^{2,3,4}

Allergic reactions occasionally develop to salicylates, the most important of which include asthma, rhinitis, urticaria and dermatitis. These usually are relieved when salicylates are discontinued, although some fatalities are reported in asthmatic patients who received aspirin in the treatment of rheumatoid arthritis.²

Corticosteroids and Corticotropin:

Cortisone and its derivatives and corticotropin in adequate dosage cause rapid suppression of pain and inflammation in rheumatoid arthritis, but when they are continued for prolonged periods undesirable to serious reactions occur in the majority of treated patients. Smaller doses usually produce more gradual improvement with fewer side effects.^{2,9,10} Corticotropin is said to be preferable to the adrenal steroids for long term therapy in the avoidance of atrophy of the adrenal cortex, although depression of pituitary function through its prolonged use is theoretically as objectionable.^{3,4}

Edema with sodium and water retention and potassium loss comprises the most frequent side effect of cortisone and hydrocortisone therapy and occurs in about half of the patients receiving 75 mgm. of cortisone or its equivalent daily over a comparatively short period. The edema is usually self limited in patients without cardiac or renal disease. The newer steroids, prednisone, prednisolone, 9- α -fluorocortisone, triamcinolone, methylprednisone and dexamethasone cause less sodium retention and potassium loss, but as their anti-rheumatic potency is increased other effects are intensified so that the major hazards of steroid therapy persist.^{10,11,16}

Frequency and severity of reactions and complications are well correlated with dosage, duration of therapy and severity of the rheumatoid disease. Side

effects are more common in women, particularly during and after the menopause. Cushingoid features of steroid therapy in most respects resemble those of natural Cushing's syndrome and consist of moon face, obesity, buffalo hump, enlarged supraclavicular fat pads, hirsutism, acne, striae and distension of the abdomen, muscular atrophy of the extremities, depression, insomnia, menstrual disturbances and edema. More serious complications of steroid therapy include hyperglycemia, hypertension, thrombophlebitis, peptic ulcer, ecchymoses, purpura, osteoporosis and pathological fracture, infections and mental abnormalities.^{2,11,12}

The hypercortisone syndrome (Chronic Hypercortisonism or Steroid Pseudorheumatism) in addition to the usual Cushingoid features is characterized by increase in fatigability, cyclic and significant emotional instability and generalized aching in bones, muscles and joints. It usually occurs in patients with rheumatoid arthritis given steroids over a prolonged period but may also occur in other patients similarly treated. Occasionally these patients may develop acute mesenchymal reactions of a diffuse nature resembling acute systemic lupus erythematosus or polyarteritis nodosa with non-specific inflammatory arteritis, peripheral neuropathy, purpura, wide spread arteriolar and small artery block with resulting ischemia, gangrene and frequently death. Most of these patients have subcutaneous nodules and high serum titers of rheumatoid factor. Similar vascular lesions were reported in patients with rheumatoid arthritis before steroids came into use but their incidence has materially increased in steroid treated patients.^{11,12}

Sudden withdrawal of corticosteroids may cause serious and severe reactions. The rebound of disease with increased intensity is frequently seen in rheumatoid arthritis patients when dosage is decreased or discontinued, due to suppression of adrenal cortical function during steroid therapy with symptoms of adrenal insufficiency on withdrawal. This creates

a special hazard in surgical patients due to electrolyte imbalance, psychic and neurological complications. The vascular complications of hypercortisonism may also initially appear when steroids are reduced or stopped and suggests that extreme fluctuations in steroid dosage may influence their development.¹⁹

The pathogenesis of peptic ulceration as a complication of steroid therapy is not clearly established, in view of the observation that a significant number of rheumatoid arthritis patients who have not received steroids develop peptic ulcer.^{2,3,4} The incidence varies from 19 to 35 percent in steroid treated patients, based on radiologic evidence, as compared to 10 percent observed at autopsy in non steroid treated patients. A higher incidence is observed in patients treated with prednisone and prednisolone in that 48 percent developed gastro-intestinal symptoms. Triamcinolone on the other hand, is said to have minimal ulcerogenic effects. Antacids when used in prednisone treated patients reduced the incidence of peptic ulcer to 3 percent but did not prevent perforation. The development of peptic ulcer seems to be directly related to the size of the prednisone dosage and length of treatment, ranging from 10 percent in patients receiving 10 mgm. daily to 47 percent in patients receiving over 60 mgm. daily. The incidence of peptic ulcer was materially reduced in patients receiving corticotropin and intramuscular steroids. Gastric ulcer occurs in greater frequency than duodenal ulcer. No appreciable changes in gastric secretory activity during steroid therapy are reported and it is proposed that steroids inhibit the healing of existing ulcers and also interfere with the protective mucosal barrier. Adequate statistics are not yet available and interpretation of results is not comparable in the various methods of treatment employed.¹⁵

Steroid induced osteoporosis is radiologically identical with senile osteoporosis and is a common complication of steroid therapy. While its mechanism is not clearly delineated it may be assumed that existing osteoporosis can be aggravated

by the catabolic actions of steroids and their effects on the parathyroid glands to cause an increased loss of urinary and fecal calcium. Pathologic fractures were reported in 21 percent of a series of 68 rheumatoid arthritis patients treated with prednisone. All but one were women over 50 years of age with rheumatoid arthritis for 10 years or longer. Fractures occurred in vertebrae, ribs, pelvic bones and femurs in about the same incidence. Aseptic necrosis of the femoral and humeral heads has been reported following systemic treatment with unusually large doses of steroids. The changes are described as Charcot like in appearance. Similar changes are reported following intra-articular steroids.^{2,3,4,22}

Muscular weakness and atrophy with weight loss frequently occur in steroid treated patients. These changes are unrelated to potassium deficiency and are probably due to interference with normal protein metabolism. It was first observed in patients treated with triamcinolone, but can be reproduced experimentally by any of the corticosteroids but is most pronounced with triamcinolone. Improvement follows when the steroid is withdrawn.¹⁴

Serious infections frequently complicate steroid therapy, and tuberculosis and virus infections are considered contra-indications to their use. Tuberculosis may be activated and deaths are reported from pneumonia, meningitis and septicemias due to staphylococci and Gram negative micro-organisms. The more severe infections usually occur in debilitated patients while ambulatory patients are commonly affected with superficial fungus infections and furunculosis. When antibiotics are used with steroids superinfection with normally non-virulent micro-organisms is not unusual. Large doses of steroids are said to alter host resistance to infection by suppression of antibody synthesis and phagocytic activity of leukocytes.^{2,3}

Posterior subcapsular cataracts have recently been reported in 38 percent of 82 patients with rheumatoid arthritis who had been on steroids for one year or

longer, compared to 2.9 percent in non steroid treated arthritic patients.^{3,4}

Although the incidence of diabetes mellitus is high in Cushing's syndrome due to adrenal tumor or hyperplasia, diabetes develops in less than 1 percent of steroid treated patients and diabetes is not considered a contra-indication to steroid therapy under carefully controlled conditions. There is no evidence that steroids can antagonize insulin activity and insulin production is normally increased with the increased synthesis of glucose and glycogen from endogenous protein by glucocorticosteroids. Most cases of decreased glucose tolerance on steroid therapy are reversible when glucocorticosteroids are returned to normal levels.¹³

Chrysotherapy: Gold salts have been used in the treatment of rheumatoid arthritis since 1929. Early studies were not well controlled in the selection of patients and dosage. Toxic reactions were reported in up to 51 percent of patients treated, requiring discontinuance of treatment in 24 percent.^{2,3,4} Toxic reactions consist of pruritis, stomatitis, urticaria, blepharitis, peripheral neuritis, erythematous and exfoliative dermatitis, anemia, thrombocytopenia, nephritis, hepatitis and encephalitis. However, no fatalities directly attributed to gold therapy have been reported in recent years.

Gold sensitivity may appear at any time during therapy, necessitating careful observation of the patient with urine and hematological examinations at the time of administering gold. Disseminated lupus erythematosus, severe kidney or liver impairment, pregnancy and severe dermatitis are contraindications to its use.^{7,8}

Gold sodium thiomalate and gold sodium thioglucose are most commonly used. They are soluble gold salts, each containing 50 percent gold. They can be administered with safety to patients with peptic ulcer, hypertension, diabetes and mental disturbances. They are reputed to offer a 20 percent better chance of major improvement or remission over other methods of treatment, as reported

in a recent 18 month double blind study in 24 clinics in Great Britain. The treated group showed significant subjective improvement over the control group although there was radiologic evidence of progressive joint destruction of equal severity in both groups. Their mode of action is not known but they probably act as inhibitors of glucosamine 6-phosphate in connective tissue.⁴

Phenylbutazone - Antpyrine Compounds: Phenylbutazone has been used clinically since 1951 and is recognized as an excellent analgesic in rheumatoid arthritis, although it has proven to be more effective in rheumatoid spondylitis and gout, due to its uricosuric action. Unfortunately it has many toxic manifestations including edema, morbilliform rash, nausea, activation of peptic ulcer, stomatitis, vertigo, nervousness, thrombocytopenic purpura and agranulocytosis. Fourteen cases of fatal leukemia and aplastic anemia have been reported following its use. About 10 percent of patients are unable to tolerate it for one or more reasons.

Edema due to sodium and water retention occurs in about 13 percent of patients, due to increased tubular absorption. Glomerular filtration and potassium excretion are not affected. It must therefore be used cautiously in patients with cardiac or renal disease. Nausea is often severe and is probably due to a central effect since antacids are not always effective in affording relief. Active liver or gall bladder disease is a contra-indication to its use. The drug has a high toxic potential and toxic effects have been reduced somewhat with a reduction in the recommended dosage. Still about 30 percent of all patients treated show some form of toxic reaction.^{2,24,25}

Toxic reactions of blood and bone marrow, including aplastic anemia, leukopenia, agranulocytosis, thrombocytopenia and purpura occur in about 2 percent of treated cases. The blood picture usually returns to normal when the drug is stopped.

Digestive disturbances of all types

occur in 12 percent of cases. The majority are of a minor nature, consisting of epigastric burning, flatulence and nausea. More severe reactions as vomiting, recurrence of peptic ulcer with hemorrhage and perforation have occurred in a few instances. Several cases of transient hepatitis were reported to occur during treatment with phenylbutazone.

Rashes and eruptions of the skin and mucous membranes occur in about 5 percent of patients. The rash is usually maculopapular or morbilliform, but in some cases may be purpuric. These reactions usually clear when the drug is discontinued. Reactions involving the mucous membranes occur less often but are more serious. On the whole the skin reactions from phenylbutazone occur less frequently and are less serious than those resulting from gold salts. However, one fatal case of exfoliative dermatitis is reported.

Antimalarial Compound: Quina-craine hydrochloride and Primaquine diphosphate were the first of the antimalarial compounds to be used in the treatment of rheumatoid arthritis but were soon discontinued because of their toxicity. Chloroquine phosphate and hydroxychloroquine sulfate were found to produce less severe side reactions. A closely related compound, Amadiaquin was found to cause hepatic damage and leukopenia. All of these have some anti-rheumatic activity in 50 to 65 percent of patients treated over a period of a few weeks to one year, and all of them cause some type of drug reaction in about one half of the patients under treatment.¹⁷

About half of the reactions observed involved the nervous or vascular systems, with vascular headaches, difficulty in visual accommodation, vestibular dysfunction, tinnitus, nervousness and mental confusion. More than one third had throbbing, unilateral headaches with or without visual auras, simulating migraine. In most instances the nervous and vascular reactions subsided after a few weeks on treatment or disappeared when treatment was stopped. It was necessary to discontinue treatment in

about 5 percent of the total group.

In some patients treated with chloroquine phosphate and hydroxychloroquine sulfate corneal deposits appeared after six months and disappeared within 3 to 6 weeks after the drugs were discontinued. Lens opacities were described in 39 percent and fundus lesions in 20 percent of patients treated with chloroquine. In other patients there were narrowing of the retinal vessels, pigmentary retinopathy and macular abnormalities, which in some cases were permanent with marked visual disability.^{3,4,27}

Gastro-intestinal symptoms occurred in about 15 percent of all patients and included anorexia, nausea, abdominal distension, heart burn, abdominal cramping and diarrhea.¹⁷

Reactions involving the skin and hair occurred in less than 1 percent of patients

receiving chloroquine compounds. Dryness of the skin, itching, urticaria, morbilliform and maculopapular eruptions, desquamating lesions and exfoliative dermatitis comprise the usual features of this group. Increased pigmentation of the skin, alopecia and graying of the hair occurred with some frequency in treated patients exposed to sunlight.¹⁷

The chloroquine compounds are only mildly to moderately effective therapeutic agents in rheumatoid arthritis and they produce fewer toxic reactions in comparison to some of the other anti-rheumatic drugs and these reactions are usually reversible when the drug is discontinued. However, further study of these compounds is necessary before adequate evaluation can be made of their alleged benefits in rheumatoid arthritis.^{2,3,4}

SUMMARY

Since rheumatoid arthritis is a disease of unknown etiology with unpredictable cyclic remissions and exacerbations, the introduction of some of the newer anti-inflammatory drugs for treatment has been responsible for many limited approaches to an understanding of its pathogenesis. While all of the drugs described have some beneficial course on the disease it must be emphasized that all of them are capable of producing

undesirable to serious side reactions which in some cases may be fatal. These drugs should be prescribed only with full knowledge of their pharmacologic actions as well as of their potentialities for toxicity. The past experiences in treatment of rheumatoid arthritis should provide further impetus toward an understanding of its causative factors and pathogenesis.^{28,29}



REFERENCES

1. Wood, P. H. N., Salicylates, *Bull. Rheumat. Dis.*, 1963, 13, pp. 297-300.
2. Rheumatism Reviews (a) Thirteenth, *Ann. Int. Med.*, 1960, 53, pp. 45-68.
3. Rheumatism Reviews (b) Fourteenth, *Ann. Int. Med.*, 1962, 56, pp. 27-45.
4. Rheumatism Reviews (c) Fifteenth, *Ann. Int. Med.*, 1963, 59, pp. 23-29.
5. Riley, H. D., Jr. and Worley, L.: Salicylate Intoxication, *Pediatrics*, 1956, 18, p. 578.
6. Singer, R. B.: Acid-Base Disturbance in Salicylate Intoxication, *Medicine*, 1954, 33, p. 1.
7. Empire Rheumatism Council Research Subcommittee: Gold Therapy in Rheumatoid Arthritis, *Bull. Rheumat. Dis.*, 1960, 11, pp. 235-238.
8. Lockie, L. M.: Gold Salt Therapy in Treatment of Rheumatoid Arthritis, *Inflammation and Disease of Connective Tissue*, W. B. Saunders Co., 1961, pp. 551-554.
9. Neustadt, D. H.: Effects of Steroids in Rheumatoid Arthritis, *Ibid.* pp. 537-543.
10. Boland, E. W.: Comparative Effects of Certain Synthetic Analogs of Hydrocortisone and Cortisone in Rheumatoid Arthritis, *Ibid.* pp. 544-550.
11. Mills, L. C.: Undesirable Effects of Steroids, *Ibid.* pp. 799-803.
12. Good, T. A.: Steroid Withdrawal Syndrome, *Ibid.* pp. 804-809.
13. Di Raimondo, V. C.: Steroid Diabetes, *Ibid.* pp. 810-811.
14. Faludi, G., Mills, L. C. and Chayes, Z. V.: Steroid Myopathy, *Ibid.* pp. 818-827.
15. Dubois, E. L., Bulgrin, J. G. and Jacobson, G.: The Corticosteroid-Induced Peptic Ulcer, *Ibid.* pp. 847-864.
16. Mills, L. C., Chayes, Z. V. and Newman, B.: Comparative Effects of Steroids on Water and Electrolyte Balance, *Ibid.* 865-867.
17. Scherbel, A. L.: Long-Term Maintenance Therapy with 4-Aminoquinoline Compounds in Rheumatoid Arthritis, *Ibid.* pp. 555-564.
18. McCarty, D. J., Jr. and Hollander, J. L.: Intra-Articular Corticosteroid Therapy, *Ibid.* pp. 565-570.
19. Slocumb, C. H.: Relative Cortisone Deficiency Simulating Exacerbation of Arthritis, *Bull. Rheumat. Dis.*, 1952, 3, pp. 21-22.
20. Bunim, J. J.: Cortisone Therapy in Rheumatoid Arthritis: A Four Year Appraisal, *Bull. Rheumat. Dis.*, 1954, 5, pp. 73-74.
21. Bernstein, C. A. and Freyberg, R. H.: Evaluation of the Status of Patients with Rheumatoid Arthritis After Five or More Years of Corticosteroid Treatment, *Bull. Rheumat. Dis.*, 1961, 12, pp. 261-262.
22. Hollander, J. L., Jessar, R. A., and Brown, E. M. Jr.: Intra-synovial Corticosteroid Therapy, A Decade of Use, *Bull. Rheumat. Dis.*, 1961, 11, pp. 239-240.
23. Ferguson, R. H., and Slocumb, C. H.: Peripheral Neuropathy in Rheumatoid Arthritis, *Bull. Rheumat. Dis.*, 1961, 11, pp. 251-254.
24. Kuzell, W. C. and Schaffarzick, R. W.: Phenylbutazone, *Bull. Rheumat. Dis.*, 1952, 3, pp. 23-24.
25. Toone, E. C., Jr.: Phenylbutazone, *Bull. Rheumat. Dis.*, 1955, 5, pp. 83-84.
26. Calkins, E., and Cohen, A. S.: Antimalarial Compounds in Rheumatoid Arthritis, *Bull. Rheumat. Dis.*, 1957, 8, pp. 149-152.
27. Von Sallman, L. and Berstein, H. N.: Adverse Effects of Chloroquine and Related Anti-Malarial Drugs on Ocular Structures, *Bull. Rheumat. Dis.*, 1963, 14, pp. 327-330.
28. Clark, G. M.: New Drugs in Rheumatic Disease, Arthritis and Rheumatism, 1962, 5, pp. 415-418.
29. Waine, H.: Management of the Arthritic Patient, Arthritis and Rheumatism, 1963, 6, pp. 83-92.

RHEUMATOID ARTHRITIS AND ORTHOPAEDIC SURGERY

WILLIAM F. HEJNA, M.D.¹

It is the purpose of this paper to bring into focus that part of the care of the patient with rheumatoid arthritis which involves an operation on his musculoskeletal system. Such focus necessarily includes a discussion of the patient's physiologic responses, surgical procedures and their indications, and the expected end result. The surgeon's contribution to the overall care of patients with rheumatoid arthritis has been directed at the comfort and function of the patient rather than at the arrest of the disease process. It is emphasized at the outset, therefore, that the team effort of an internist, surgeon, physiatrist, physiotherapist, and occupational therapist, makes a more comprehensive approach to this multifaceted problem.

GENERAL CONSIDERATIONS

Primary among the aims of surgery in these cases is the relief of pain, and improvement in the function of joints. Although the pain may be due to myositis, neuritis, or fasciitis, it is frequently caused by destroyed joints and the attendant limb attitude, or synovial proliferation and inflammation. In the latter situations, surgery may offer significant relief of pain. Procedures designed to improve function are variable and may include arthroplasty of bilaterally ankylosed temporomandibular or hip joints in one patient, or fusion of a thumb or digit joint in another. It must be borne in mind that the motion of a particular joint or the total capability of the individual, is assessed on the basis of the degree of involvement of that joint and parts affecting its function, the severity of his generalized disease, and the patient's adaptation to his deformities. It should not be compared to the normal

individual. Of secondary consideration, though perhaps no less important, is the aim to prophylaxis. For example, a proliferating synovium may act as a factor in eventual rupture of tendons, or may by its presence hasten pannus formation and joint destruction. Such diseased tissue should be removed. Further, contractures or luxations about a joint may adversely affect another joint in the limb, and preventive surgery may be indicated.

Unless the patient is in crisis, the decision to operate should not be dictated by clinical activity of the disease or the sedimentation rate. Particular evaluation is however warranted, since rheumatoid arthritis in its sundry forms may be a protean disease. Anemia, for example, is common and efforts should be made to correct this prior to surgery. Fluid and nutritional imbalances, and cardiopulmonary and renal abnormalities must also be recognized and safely controlled. An additional problem may be presented by systemic therapy which the patient has received. Phenylbutazone^{1,2}, and salicylates³ have pertinent side effects but rarely are a contraindication to surgery, whereas patients who have received systemic corticosteroid therapy present a more particular problem. The additional

From the Department of Orthopaedic Surgery, Division of Surgery

¹ Adjunct Attending Surgeon, Presbyterian-St. Luke's Hospital; Clinical Instructor, University of Illinois College of Medicine

stress created by surgery and an anesthetic in a patient whose adrenal glands are suppressed by exogenous steroids, may develop adrenal insufficiency with shock. At this institution, this complication has been avoided by the management of such patients as follows: One hundred mg. of cortisone is administered intramuscularly the day before surgery, 100 mg. is then given every 8 hrs. the day of surgery, and 50 mg. every 8 hrs. on the day following surgery. Oral cortisone is then given in decreasing amounts over 4-5 days returning to the patient's pre-operative maintenance dose. In addition, 150 mg. of hydrocortisone is given intravenously during the operation. It is mandatory that maximum precautionary efforts in aseptic technique and operating room policies regarding sepsis be made, since infection is more difficult to control and physiologic responses such as fever and leukocytosis can be masked. Long term steroid therapy may also cause osteoporosis, and rarely aseptic necrosis in the bone. These points are of significance when the bone tolerance to metal is critical, as in the use of fixation devices and endoprostheses.

Although much progress has been made in the field of anesthesiology, the anesthesiologist must be apprised of such factors as arthritis in the cricoarytenoid and temporomandibular joints, and in the cervical spine, so that problems of intubation, ventilation, and oxygenation, are more easily handled. The argument in favor of field and nerve block anesthesia to minimize the physiologic stress is frequently countered with the fact that ventilation is better controlled under general anesthesia.

In the pre-operative and post-operative periods, it is imperative that appropriate physical methods be utilized. For example, graduated resistance exercises maintain power; passive exercises and splinting prevent contractures and maintain the range of motion in joints; and braces, crutches, canes, and other assistive devices, aid the individual in his total capability.

PROCEDURES (EXCLUDING HAND AND FOOT)

Synovectomy

This procedure is most frequently carried out upon the knee and wrist to relieve pain, restore function, and to remove a focus of diseased tissue which causes local joint destruction.^{4,5} London⁶ reported results in 34 instances of synovectomy of the knee and stated that the procedure is of definite value for the relief of pain. The end result was in part determined by the state of the articular cartilage at the time of operation. On the dorsum of the wrist synovectomy is indicated when there is proliferation, since synovitis and tenosynovitis abet volar luxation of the carpus and are additive factors in the degeneration and spontaneous rupture of tendons.⁷ On the volar aspect, hyperplasia of the synovium under the carpal ligament may cause pressure on the median nerve and necessitate decompression by section of the ligament and synovectomy. Further, synovial membrane may dissect well into the calf and present as symptomatic cysts.⁸ Such cysts may begin as outpouchings of the synovium of the joint or may in other areas represent markedly enlarged bursae (Figure 1). When these are sufficiently troublesome to the patient they should be excised.

Arthroplasty

Reconstruction of a joint can be accomplished by merely reshaping the bony contours, or by interposing a material such as fascia, skin, or metal. It can also be done by removing the destroyed bone and replacing it with a metal part. These operations are performed in order to maintain or regain painless motion and to promote stability.

Although some controversy exists regarding the efficacy of vitallium mold arthroplasty in the rheumatoid hip, some authors advocate its use and state that significant improvement is made in these patients.^{9,10,11} For example, Law^{9b} reported that in 26 cases, (ten with unila-

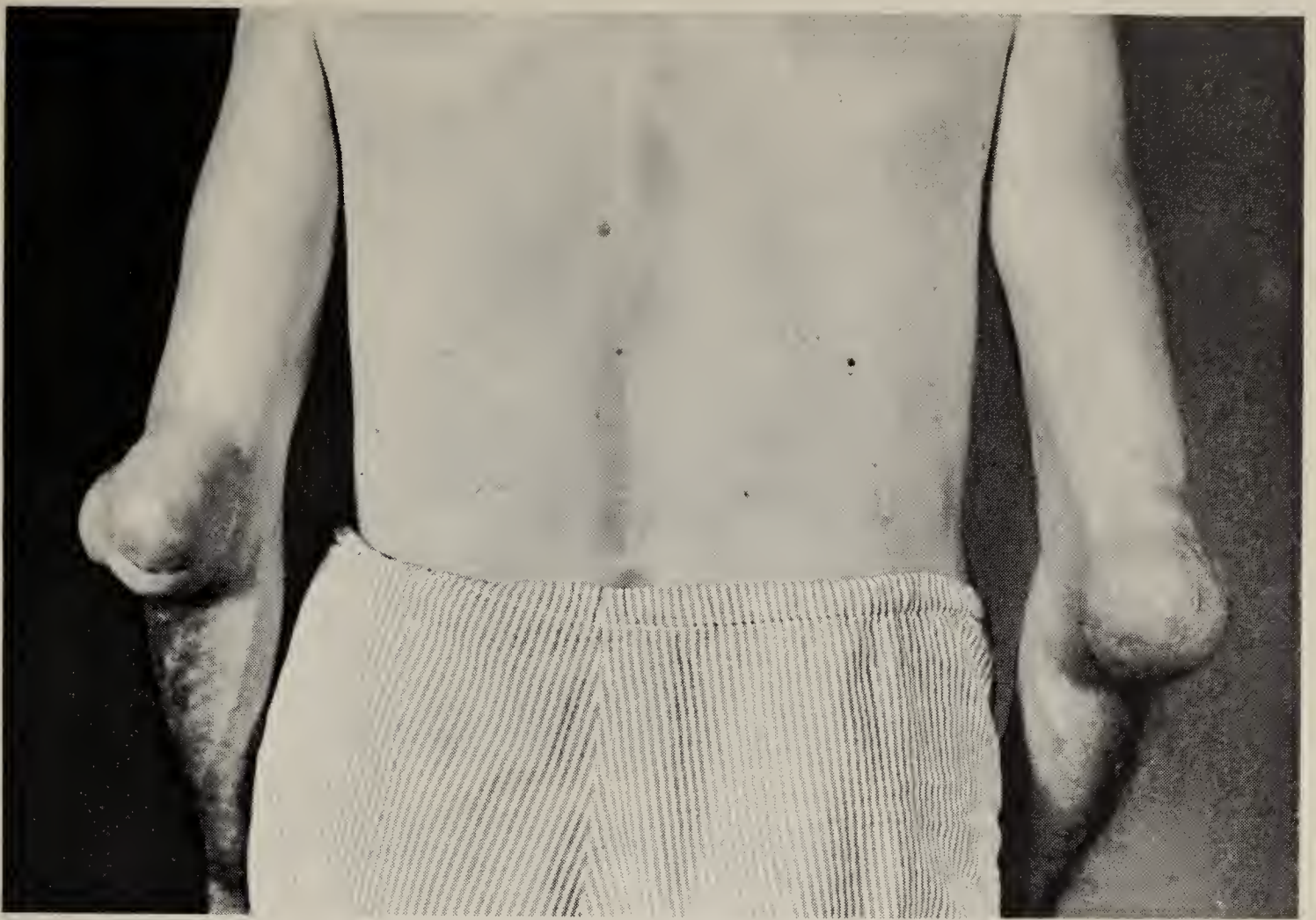


Fig. 1.—The patient was a 60 year-old man with known rheumatoid arthritis for several years. The masses about the elbows had increased in size, were tender, and caused him embarrassment. They were found to contain 2 or 3 large rheumatoid nodules and a synovial lined sack with approximately 60cc. of cholesterol crystals and detritus.

teral and 16 with bilateral involvement) satisfactory results were obtained in all but four. He stressed that revisions are frequently necessary and should not be deterrents to such reconstruction. Generally, a better end result may be predicted if the pre-operative range of motion and power about the hip are good, and the patient is well motivated. Post-operatively, the patient must tolerate a three to six month period of non-weight bearing, during which a program of active exercises is carried out. Figure 2 shows such a mold in place.

Arthroplasty of the elbow provides a rewarding result particularly when there is bilateral involvement, since a patient with such ankylosis cannot feed or dress himself, or carry out normal hygiene. Motion is gained by excising the radial head and reshaping the remaining joint surfaces while protecting the ulnar nerve. Active exercises, whirlpool, and other

physical measures are begun about one week post-operatively, and the patient is encouraged to use the limb in a graduated fashion. He rarely objects the 15° of mediolateral instability which invariably occurs. (Figure 3)

Replacement prostheses have been developed for the hip, knee, and humeral head, and although the first of these is commonly used in other lesions, none has found widespread use in rheumatoid arthritis.

Osteotomy

The most common fixed attitude of the knee which incapacitates the patient, is a flexion contracture with or without valgus. Traction, splinting, and other measures frequently fail to correct this deformity, and surgery is indicated. If no valgus exists, a soft tissue release including section of the capsule and gastrocnemius heads may allow adequate



Fig. 2.—The head of the femur and the acetabulum were thoroughly reshaped and the appropriate sized mold is interposed. Early motion was begun while the patient was in a suspended support.



Fig. 3.—The patient had rheumatoid arthritis with ankylosis of the right elbow for 35 years. She developed progressive changes and pain in the left elbow and an arthroplasty was carried out on the right. Motion increased to 115° of flexion, minus 40° of extension and 25° of pronation—supination. She has fair to good power in the biceps and triceps, and no pain.

extension. When valgus is present, it may be necessary to perform an osteotomy of the upper tibia or of the supracondylar area of the femur.¹² If the bone is cut, blade plate fixation is used and knee motion is begun early. Such a procedure realigns weight bearing and places the existing motion within a serviceable range.

Osteotomies have also been done in which the head and neck of the femur are resected.^{13,14} Such "pelvic support" procedures eliminate pain while retaining some motion. (Figure 4) Although the reconstruction is not anatomical, and some limp persists, the patient is usually appreciative of this alternative to bilaterally ankylosed hips. Other resections which offer great relief of pain with little functional loss are those of the distal ulna¹⁵ and distal clavicle.

A severe degree of kyphosis may be present in the ankylosed spine of a patient with rheumatoid ankylosing spondylitis. Wedge resection osteotomy of the posterior elements of the spine can be carried out in the lumbar region thereby allowing the patient to stand erect, to look forward, and to walk with greater ease and safety.^{16,17} It has also been done in the cervicle spine when chewing and swallowing were difficult, however, the surgical risk is greater.¹⁸

Arthrodesis

The fusion of a joint eliminates pain and creates stability. The loss of motion, however, may be incapacitating when the contralateral joint is involved or when another major joint in the same limb is involved. Arthrodesis is therefore indicated when a) there is monarticular involvement, especially in the knee; b) there is a painful fibrous ankylosis and a motion producing procedure is not deemed feasible; c) when the joint under consideration normally has a small range of motion, such as the subtalar joint; and d) when it is done to gain a mechanical advantage such as fusion of the radiocarpal joint to place the finger tendons in the optimal range of excursion. The prime criterion of a successful



Fig. 4.—Following this procedure, the hip is usually maintained in flexion while the soft tissues heal. Early motion is begun and the patient may ambulate six weeks post-operatively.

arthrodesis of any joint remains the x-ray evidence of bridging trabeculae, and achievement of the clinical aim.

Many joint fusion techniques have been devised. Among those which have gained wide usage is the method of Charnley,¹⁹ in which compression of the bone ends is accomplished. The incidence of successful knee arthrodesis for example is high, and the patient is usually allowed to bear full weight in 3 months.

THE RHEUMATOID HAND

There are numerous factors which may contribute to the rheumatoid hand attitude. Destroyed joint surfaces and peri-articular soft tissues stretched by effusion and synovial hyperplasia are easy prey to muscle imbalances, tendon ruptures, dislocations, and intrinsic muscle contracture. Most commonly the so called "intrinsic plus" hand is seen, in which the metacarpophalangeal joints are flexed, the interphalangeal joints are extended, and the digits are in ulnar deviation. The thumb is drawn into the palm by contracture of the short adductor and the thenar group, and the distal thumb joint may be in hyperextension. Tension in such fingers can be relieved by shortening the metacarpals at their heads or bases, and by the section of contractures.²⁰ In the thumb a more extensive soft tissue release is usually

necessary and frequently must be combined with a fusion for stability.^{20,21} Ulnar drift is best corrected by a shortening of the metacarpals combined with section of the lateral bands, and relocation of the long extensor tendons on top of the knuckles. Synovectomy of the metacarpophalangeal joint should also be done when there is hyperplasia and effusion, since this process promotes the myositic intrinsic contractures and joint luxations.

A second common attitude in the digits is the boutonniere deformity with marked flexion at the proximal interphalangeal joint. This is caused by a split in the dorsal hood allowing the lateral bands to slip volarward and act as flexors. When of short duration, repair of the hood may be done, however, when the deformity has been present for a long time or when associated with long standing luxation, arthrodesis in a suitable position is the procedure of choice.

Spontaneous rupture of tendons most commonly occurs in the long extensors of the fingers and may be due to a combination of poor nutrition, invasion by rheumatoid granulation tissue, and abrasion by bone, especially at the distal ulnar-carpal joint. Repair of the tendon with or without a graft is indicated, and is frequently combined with resection of the distal ulna and synovectomy.²²

Currently under study is the use of endoprostheses in the metacarpophalangeal and interphalangeal joints.²³ These metal hinge joints are said to provide motion, decrease pain, and increase

stability. The primary indications are gross joint destruction or ankylosis, palmar dislocations at the metacarpophalangeal joints, and persistent ulnar drift when the extensor mechanism is intact and the other finger joints are functional. The insertion of such prostheses may also be combined with relocation of the tendons over the knuckles and synovectomy. Other work including transplantation of whole joints has been done, however, major problems in this area have included revascularization, denervation, and the maintenance of the proper relationship of component parts.²⁴

THE RHEUMATOID FOOT

Prominent among the complaints of the rheumatoid patient is pain in the feet where the punishment of weight bearing is unending. The hallux valgus with its bunion, and the dropped metatarsal heads with cocked up toes, remind the patient of his ill fitting shoes with each step. When conservative measures fail, these deformities may be improved by adequate resection of the phalangeal bases plus the metatarsal heads, and imbrication of the medial first metatarsophalangeal joint capsule.¹¹ Although the foot is slightly shortened, push off and pain are improved, and the patient no longer walks on his heels. Amputation of the forefoot has been recommended when these deformities are severe, however, this must be considered a last stage procedure.²⁵ Finally, involvement of the subtalar joint with pain on weight bearing is best treated by triple arthrodesis.

CONCLUSION

Orthopaedic surgery in rheumatoid arthritis has had its infancy during the past fifteen years. Improved techniques, modern anesthesia, and a somewhat better understanding of the course of the disease has aroused enthusiasm which

will most certainly bring new levels of achievement and benefit to the patient. It remains the obligation of those caring for such patients to know the available methods, to apply them correctly, and to critically evaluate.



REFERENCES

1. Mason, R. M., and Steinberg, V. L.: Long Term Use of Phenylbutazone in Rheumatoid Arthritis. *Brit. Med. Jour.* 2:828, 1960.
2. Steinbrocken, O., and Argyras T. G.: Phenylbutazone as a Therapeutic Agent in Rheumatic Diseases: Its Present Status *Arthritis Rheum.* 3:368, 1960.
3. Hart, F. D.: Analgesics in Rheumatic Disorders. *Brit. Med. Jour.* 1:1265, 1960.
4. Swett, P. P.: A Review of Synovectomy. *J. Bone & Joint Surg.* 20:70, 1938.
5. Law, W. A.: Symposium on the Management of Rheumatoid Arthritis. *J. Bone & Joint Surg.* 34B:148, 1952.
6. London, P. S.: Synovectomy of the Knee in Rheumatoid Arthritis. 37B:392, 1955.
7. Potter, T. A., and Kuhns, J. G.: Rheumatoid Tenosynovitis: Diagnosis and Treatment. *J. Bone & Joint Surg.* 40A:1230, 1958.
8. Maudsley, R. H., and Arden, G. P.: Rheumatoid Cysts of the Calf and their Relation to Baker's Cysts of the Knee. *J. Bone & Joint Surg.* 43B:87, 1961.
9. Law, W. A.: Late Results of Vitallium Mold Arthroplasty of the Hip. *J. Bone & Joint Surg.* 44A:1497, 1962.
Post-Operative Study of Vitallium Mold Arthroplasty of the Hip Joint. *J. Bone & Joint Surg.* 30B:76, 1948.
10. Aufrance, O. E.: *Constructive Surgery of the Hip.* C. V. Mosby Co., 1962.
11. Smith Petersen, M. N.: Arthroplasty of the Hip; A new Method. *J. Bone & Joint Surg.* 21:269, 1939.
12. Clayton, M. L.: Surgery of the Lower Extremity in Rheumatoid Arthritis. *J. Bone & Joint Surg.* 45A:1517, 1963.
13. Batchelor, J. S.: Excision of Both Femoral Heads in a case of Ankylosing Spondylitis. *Proc. Roy. Soc. Med.* 35:224, 1941-42.
14. Milch, H.: The Resection-Angulation Operation for Hip Joint Disabilities. *J. Bone & Joint Surg.* 37A:699, 1955.
15. Darach, W.: Habitual Forward Dislocation of the Head of the Ulna. *Ann. Surg.* 57:928, 1913.
16. Smith Petersen, M. N., Larsen, C. B., and Aufrance, O. E.: Osteotomy of the Spine for Correction of Flexion Deformity in Rheumatoid Arthritis. *J. Bone & Joint Surg.* 27:1, 1945.
17. Adams, C.: Technique, Dangers, and Safeguards, in Osteotomy of the Spine. *J. Bone & Joint Surg.* 34B:226, 1962.
18. Freeman, G.: Correction of Severe Deformity of the Cervicle Spine in Ankylosing Spondylitis with the Halo Devic. *J. Bone & Joint Surg.* 43A:547, 1961.
19. Charnley J.: Positive Pressure Arthrodesis of the Knee Joint. *J. Bone & Joint Surg.* 30B:478, 1948.
20. Bunnell, S.: Surgery of the Rheumatic Hand. *J. Bone & Joint Surg.* 37B:759, 1955.
21. Clayton, M. L.: Surgery of the Thumb in Rheumatoid Arthritis. *J. Bone & Joint Surg.* 44A:1376, 1962.
22. Vaughn-Jackson, O. J.: Rheumatoid Hand Deformities Considered in the Light of Tendon Imbalance. *J. Bone & Joint Surg.* 00B:9999, 9999.
23. Flatt, A.: Restoration of Rheumatoid Finger Joint Function. *J. Bone & Joint Surg.* 43A:753, 1961.
24. Entin, M. A., Alger, J. R., Baird, R. M.: Experimental and Clinical Transplantation of Autogenous Whole Joints. *J. Bone & Joint Surg.* 44A:1518, 1962.
25. Law, A.: Symposium of the Management of Rheumatoid Arthritis. *J. Bone & Surg.* 34B:148, 1952.

ASEPTIC NECROSIS OF BONE COMPLICATING CORTICOSTEROID THERAPY FOR PEMPHIGUS VULGARIS

JAMES C. LOWE, II, M.D.¹

Of the many known complications of corticosteroid therapy, aseptic necrosis of bone is one of the more unusual and one which went unnoticed for some time. The first evidence of this phenomenon was noted in 1952 with the next case reported in 1957.^{1,2} In 1963, Sutton et al reviewed the current published literature of 70 cases.³⁻⁸ The pathogenesis of the process is unknown.

We have recently observed a patient with this complication affecting a bone area not previously reported.

M.J.T., a 36 year old Negress, was admitted for evaluation of aching painful knees of four months duration. This pain was present intermittently since 1958 and at that time x-rays of both knees were negative and joint aspiration was unsuccessful. Also Uric acid levels and latex fixation tests were normal. Four months before her admission, in September 1963, she noted a marked increase in symptoms which were progressive until admission. The knees were swollen and sometimes warm. The aching pain of the knees decreased when she was up and around. Sometimes there was tibial pain as well. As an out-patient, joint aspiration was again unsuccessful and because x-rays revealed an unusual abnormality of the bones surrounding the knee joints she was admitted for further evaluation.

In 1950 she experienced the onset of episodes of painful vesicular and bullous eruptions of arms, legs, trunk, and oral cavity. She was first seen at Presbyterian-St. Luke's Hospital in 1956 where a skin biopsy confirmed the clinical impression of pemphigus vulgaris. Since 1957, she had been continuously on corticosteroid therapy until eight months before ad-

mission. In 1958 and 1959 she received 100 mg. of prednisone daily. In 1959 and 1960 she received 24 mg. of triamcinalone daily, and in 1960 through 1962 she again received varying doses of prednisone. In addition, she was treated by intravenous adrenocorticotrophic hormone (ACTH) drip on most of her hospital admissions between 1956 and 1963. In the year prior to admission she had no recurrence of her pemphigus vulgaris and steroid therapy was discontinued completely eight months prior to the present admission.

The family history was unremarkable. At age 11 she had an appendectomy, in 1951 she had pneumonia and in 1960 a left breast biopsy which was benign. There was no history of acute or repetitive trauma. The patient was Grav II Para I Ab-1.

Physical examination revealed a well developed, well nourished, well appearing Negress with scattered areas of increased pigmentation over the extremities, the trunk, and the oral cavity. No evidence of active pemphigus was seen. The extremities were all normal except for the knees. Both were slightly swollen, slightly tender to palpation but without heat. There was full motion with only mild discomfort. The physical findings were otherwise within normal limits. The temperature was 98.8°F, the pulse 84 and the respirations 28. The blood pressure was 100/60.

From Division of Medicine

¹ Chief Medical Resident, Health Center Inpatient Physician, Presbyterian-St. Luke's Hospital; Instructor in Medicine, University of Illinois College of Medicine

The hemoglobin was 13.5 gms%; the hematocrit 42% and the white cell count 9,000 mm with a normal differential. The urinalysis was normal. The sedimentation rate was 30 mm/hr (Wintrobe); the blood urea nitrogen 10 mg% and the uric acid on two occasions 6.6 and 6.8 mg%. The two hour post-prandial blood sugar was 89mg%. The Kahn and Wasserman tests were negative. C-reactive protein was negative. Three LE preparations were negative. Serum protein electrophoresis was normal. Latex fixation titer was 1:640. Sheep cell agglutination test was negative. The alkaline phosphatase was 2.2 Bessy-Lowry units, the calcium 9.9 mg% and the phosphorus 3.3 mg%. Films of the chest were negative. Films of bones and joints were normal except for the femur and tibia adjacent to both knee joints.

There was a peculiar wavy appearance of the external surface of the bone with the cortex irregularly thickened and with an irregular, dense, cystic-like medullary sclerosis. (Fig. 1 and Fig. 2.) These changes were felt to be compatible radiologically with sickle cell anemia, myelofibrosis, Gaucher's disease, or aseptic necrosis.

Skin tests for tuberculosis, histoplasmosis, coccidioidomycosis, and Blastomycosis were negative. Aspiration of the joint space was again unsuccessful. Accordingly, at surgery there was performed an open biopsy of the synovium of the left knee and a trephine biopsy of bone from the left medial femoral condyle. The microscopic diagnosis was chronic synovitis and revascularizing aseptic necrosis of bone. (See Fig. 3 and Fig. 4.) The patient improved at bedrest.

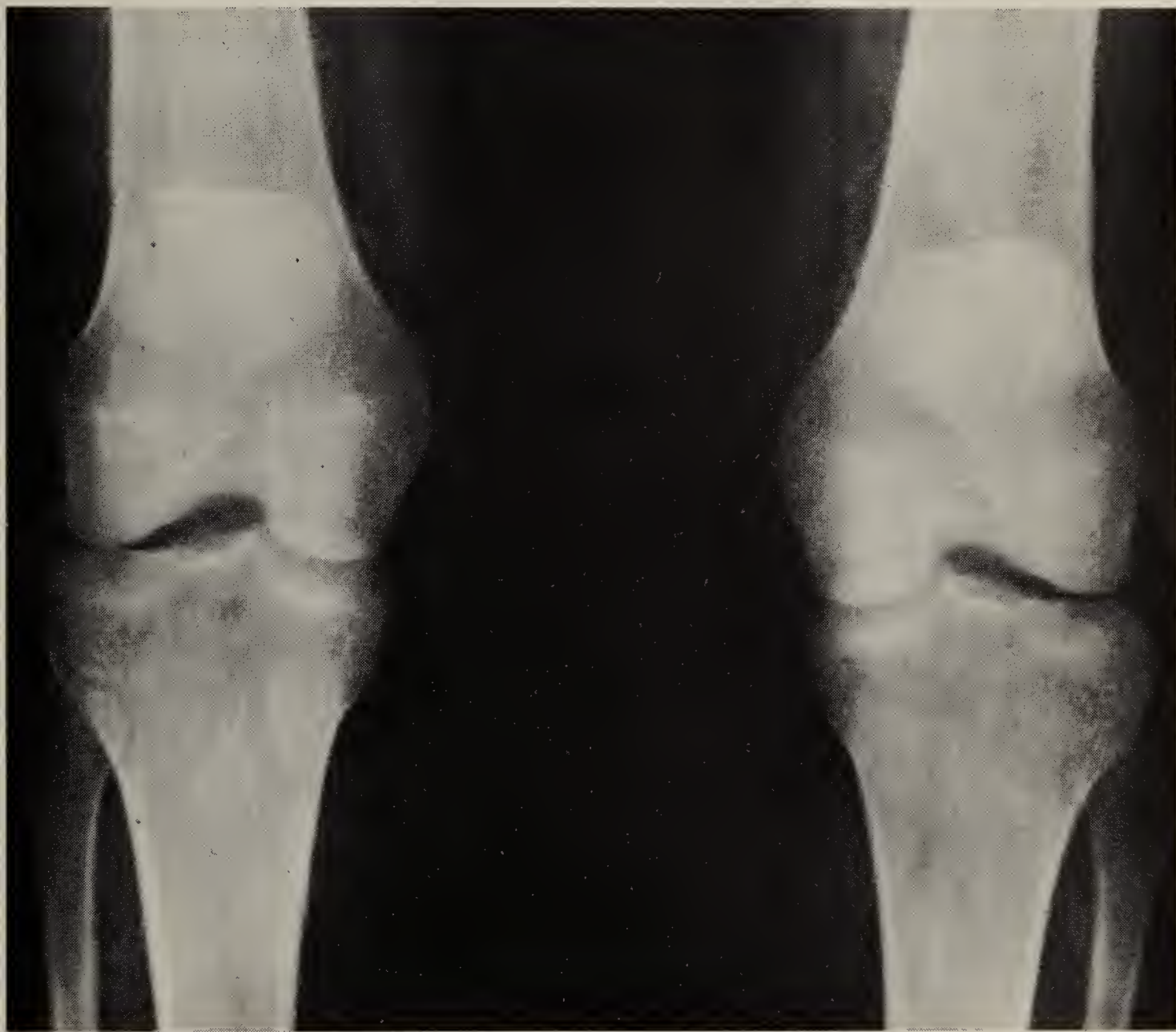


Fig. 1.—X-ray films of both knees (1958). Normal.

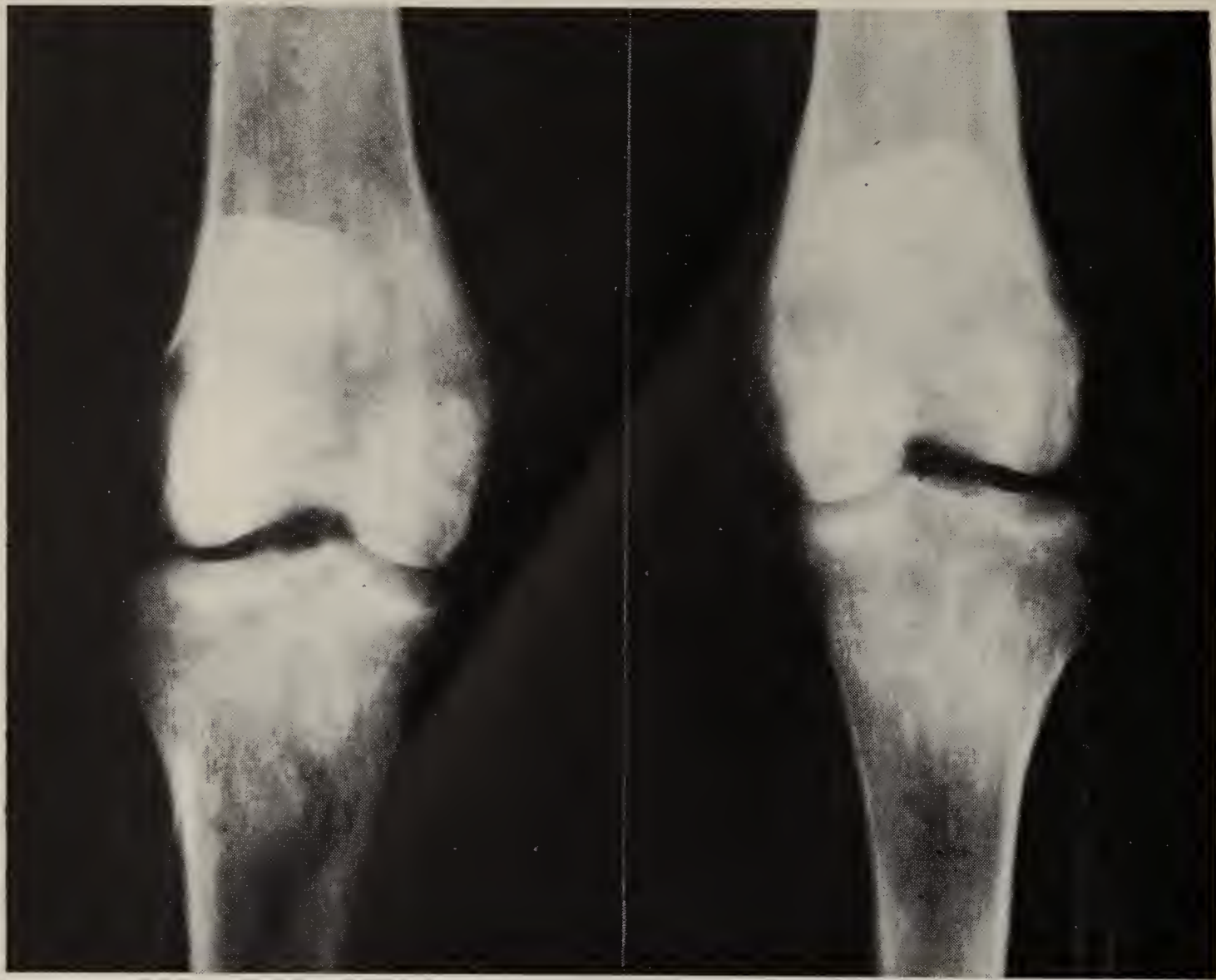


Fig. 2.—X-rays films of both knees taken September 1963. Note the wavy irregularity of the surface of the cortex and the irregular thickening of the rertex. There is irregular, dense, cystic sclerosis of the medullary area.



Fig. 3.—Biopsy of left medial femoral condyle. Microscopic diagram revascularizing aseptic necrosis of bone.

COMMENT

Aseptic necrosis of bone is defined as a degenerative necrosis of osseocartilaginous tissue without infection. It is most often seen in children where many eponyms are attached. As a primary disease in adults, it is called Chandler's disease. However most often in adults, it is a secondary process with the most common site of involvement being the femoral head. The humeral head is also involved fairly frequently. This lesion is usually due to interruption of the blood supply to the head of the femur due to single or repetitive trauma to the area. It is also seen in the hemoglobinopathies⁹, Gauchers disease¹⁰, caissons disease¹¹, and rheumatoid arthritis with bony abnormalities of the joint (protrusio acetabuli)⁴.

The symptoms are pain in the affected area, usually with limitation of motion.

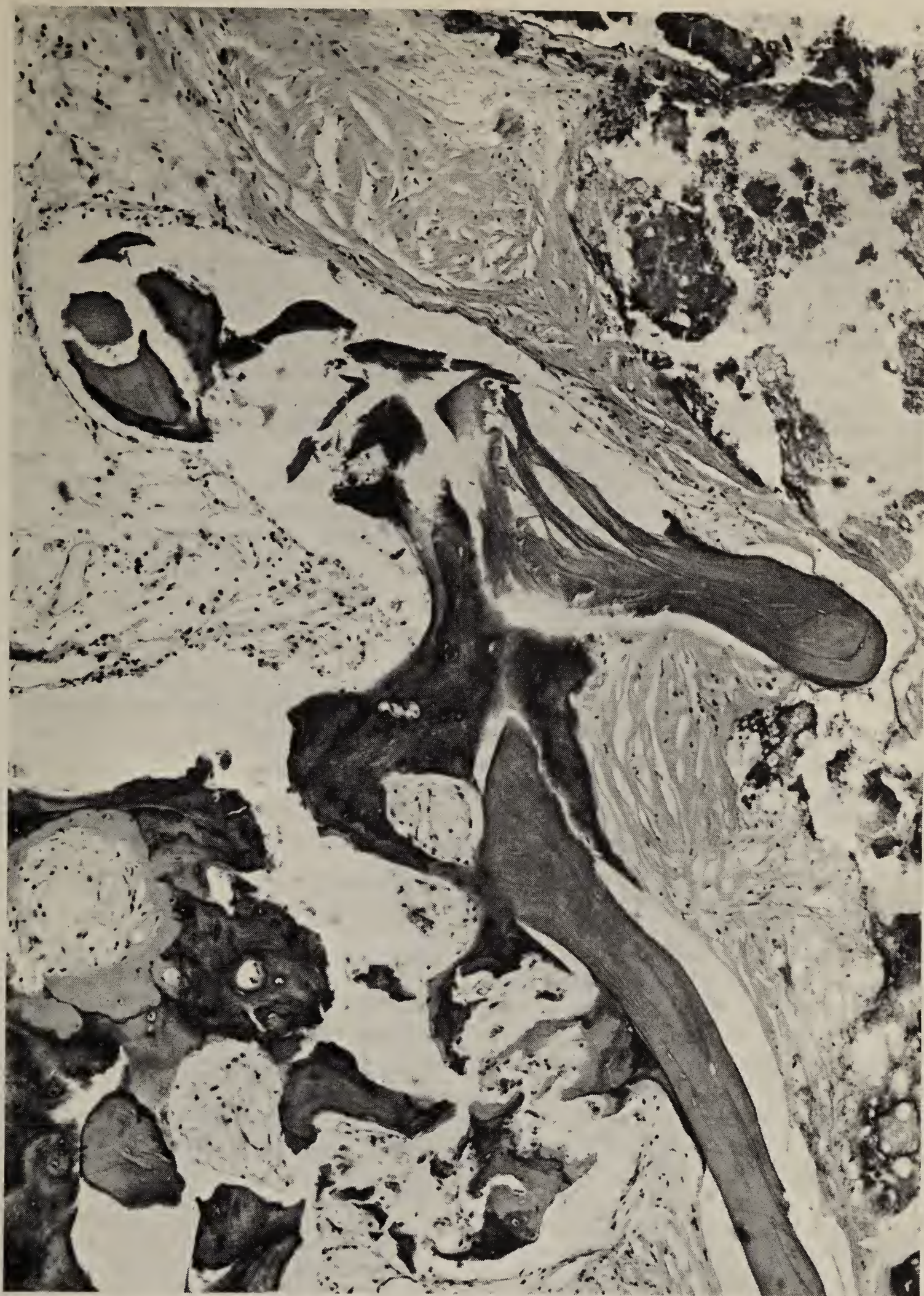


Fig. 4.—Biopsy of left medial femoral condyle. Photomicrograph revascularizing aseptic necrosis of bone.

The pain generally begins insidiously and is progressive in intensity. A few cases are reported of sudden onset of pain. Symptoms vary in duration from months to years. During the acute necrotic phase there are no abnormal laboratory studies and x-ray films are normal. The first x-ray changes appear during the healing phase of revascularization. Increased density and cystic changes are noted. During this period of healing, the bone is soft and complete rest may prevent serious bone and joint derangement.

The pathogenesis of aseptic necrosis of bone as a complication of corticosteroid therapy is unknown. There is, in fact, no proved relationship. In some of the diseases for which steroids are administered, aseptic necrosis of bone may occur as part of the primary process. Most notable among these are rheumatoid arthritis and systemic lupus erythematosus. However, in 31 of the 70 reported cases, this lesion has appeared in diseases in which aseptic necrosis of bone is not seen. These include pemphigus vulgaris, osteoarthritis, eczema, sarcoidosis, idopathic purpura, subacute bacterial endocarditis, erythema multiforme, gout, multiple sclerosis, uveitis, psoriasis, subacute hepatitis, tuberculosis, premycotic erythrodermia, and rheumatic fever. (See Table 1) In none of the 70 reported cases has there been any history of trauma. Therefore, there is more than a chance association of aseptic necrosis of bone with steroid therapy in these patients who have nothing else in common. The incidence of aseptic necrosis of bone in all patients receiving corticosteroid therapy is obviously quite low. Exact figures are difficult to obtain, however, because this complication has not been looked for and because it occurs as a primary manifestation in rheumatoid arthritis and systemic lupus erythematosus. No figures for the incidence of aseptic necrosis of bone in the general population are available.

No relationship has as yet emerged between dosage or duration of treatment. It does seem clear that revascularization usually does not begin to occur until

steroids are discontinued. Therefore, the x-ray change may not appear until that time. This indeed was the case with the present patient.

In the 70 reported patients only the femoral and humeral heads were involved in all but one case where the metacarpals were involved. The case reported herein is the first to note involvement of the bone adjacent to the knees. It is also unique with the bilateral symmetry of the lesion.

Although the mechanism of action of corticosteroid compounds in producing this lesion is not known and the incidence is low, nevertheless, all patients to whom corticosteroids are given must be observed for the possibility of aseptic necrosis of bone since rest and cessation of steroid therapy, where feasible, may well prevent crippling bone and joint disease.

TABLE I
Diseases in Which Aseptic Bone Necrosis
Due to Corticosteroids Have Been Reported

Rheumatoid Arthritis.....	34
Systemic Lupus Erythematosus.....	13
Pemphigus.....	3
Osteoarthritis.....	3
Eczema.....	2
Sarcoidosis.....	2
Idiopathic Purpura.....	2
Subacute Bacterial Endocarditis.....	1
Erythema Multiforme.....	1
Gout.....	1
Multiple Sclerosis.....	1
Uveitis.....	1
Hemolytic Anemia.....	1
Psoriasis.....	1
Sub-acute Hepatitis.....	1
Tuberculosis.....	1
Premycotic Erythroderma.....	1
Rheumatic Fever.....	1

SUMMARY

A case is reported of aseptic necrosis of bone involving the tibia and femur adjacent to both knee joints in association with prolonged corticosteroid administration for pemphigus vulgaris. This is the first reported case in which bilateral symmetrical involvement of the knees occurred.

This disease usually affects only femoral and humeral heads, and its pathogenesis

is unknown. There is no demonstrable correlation between dosage or duration of therapy with corticosteroids. Healing most often occurs when steroids are withdrawn. Complete rest is important during healing.

As an important, though rare complication of corticosteroid therapy, it's early recognition and treatment may prevent bone and joint deformity.



REFERENCES

1. Heimann, W. G. and Freiburger, R. H.: Avascular Necrosis of the Femoral and Humeral Heads after High-Dosage Corticosteroid Therapy. *New England J. Med.* 263:672, 1960.
2. Pietrogrande, V. and Mastromarino, R.: Osteopatia da prolungata trattamento cortisonico. *OrtopTraumat* 25:791, 1957.
3. Sutton, R. D., Benedek, T. G. and Edwards, G. A.: Aseptic Bone Necrosis and Corticosteroid Therapy. *Arch. Int. Med.* 12:594, 1963.
4. Edstrom, G.: Destruction of Hip Joints in Rheumatoid Arthritis During Long-Term Steroid Therapy. *Acta Rheum. Scand.* 7:151, 1961.
5. Dubois, E. L., and Cozen, L.: Avascular Bone Necrosis Associated with Systemic Lupus Erythematosus. *J.A.M.A.* 174:966, 1960.
6. Siemsen, J. K., Book, J. and Meister, L.: Lupus Erythematosus and Avascular Bone Necrosis: A Clinical Study of 3 cases and Review of the Literature. *Arthritis Rheum.* 5:492, 1962.
7. Sweetnam, D. R., Mason, R. M. and Murray, R. O.: Steroid Arthropathy of the Hip. *Brit. Med. J.* 1:1392, 1960.
8. Meyer, T., Golter, L. E. and Hawley, C.: Avascular Necrosis of Bone Following Systemic Steroid Therapy. *Radiology* 80:422, 1963.
9. Sherman, M.: Pathogenesis of Disintegration of the Hip in Sickle Cell Anemia. *Southern Med. J.* 52:632, 1959.
10. Davies, F. W.: Gauchers Disease in Bone. *J. Bone Joint Surg. (Brit.)* 34B:454, 1952.
11. Poppel, M. H. and Robinson, W. T.: The Roentgen Manifestations of Caisson Disease. *Amer. J. Roentgen* 76:74, 1956.

REVIEW ARTICLE

CYTOTOXIC ANTIBODIES:

Their Significance in "Autoimmune" Disease

ROBERT E. DEDMON, M.D.¹

Autoimmune disease has been defined (1) as a "condition in which structural or functional damage is produced by the action of immunologically competent cells or antibodies against normal components of the body." A number of diseases, including acquired hemolytic anemia, Hashimoto's thyroiditis, systemic lupus erythematosus, rheumatoid arthritis, ulcerative colitis, lupoid hepatitis, allergic encephalomyelitis, orchitis, uveitis and adrenalitis, and certain types of glomerulonephritis, are thought to be of this variety. The list seems to increase daily! One of the features common to a number of these disorders is the presence of circulating antibodies (antinuclear factors, L-E cell factors, hemagglutinating and complement-fixing antibodies, rheumatoid factors and cytotoxic antibodies) directed against normal tissue antigens. Cytotoxic antibodies are the object of this discussion. Although studies of acquired hemolytic anemia have provided much of the basis for our understanding of cytolytic immune reactions, these will not be reviewed. Instead, attention will be given to *in vitro* studies of the nature of immune cytolysis in human and experimental diseases, namely: rheumatoid arthritis, Hashimoto's thyroiditis, ulcerative colitis, systemic lupus erythematosus, and experimental allergic encephalomyelitis. The significance of humoral antibodies in the homograft reaction and tumor immunity has been reviewed by others (6-11) and will be discussed only insofar as it applies to immune cytolysis in general.

HUMORAL ANTIBODIES

Circulating antibodies may be studied either by standard serologic reactions *in vitro* (e.g. hemagglutination, complement-fixation, and the precipitin re-

action) or by their biologic effect. Two ways of evaluating the latter are inhibition of a graft (e.g. tumor) by incubation of donor cells with hyperimmune serum prior to grafting or by demonstration of cytotoxic effects *in vitro*⁶. In an attempt to elucidate the events occurring in antibody mediated cell lysis many investigators have turned to tissue culture studies⁴. Such studies in transplantation and autoimmune disease are relatively recent, but were preceded by earlier investigations of tumor immunity.

From the Division of Medicine and Department of Microbiology

¹ Adjunct Physician, Presbyterian-St. Luke's Hospital; Instructor in Medicine, University of Illinois College of Medicine
Present Address—211 N. Commercial Street, Neenah, Wisc.

In 1911 Lambert and Hanes¹² found that sera from animals immunized against heterologous sarcomas inhibited the growth of these cells in tissue culture. However, one of the problems in applying such studies to tumor immunity has been that extensive cross reactions occur, and it has not been possible to distinguish immunologically distinct cell types in tissue culture investigations^{13,14}. One other problem is evaluation of the significance of circulating antibodies in the pathogenesis of cell damage *in vivo*. For example, hemagglutinating antibodies can be removed by absorption from some immune sera without affecting ability of these sera to shorten survival of a tumor graft⁶. In the case of certain sarcomas¹⁵ antiserum causes enhancement of growth in the host.

In the area of autoimmune disease, sera from patients with rheumatoid arthritis are toxic to normal synovial cells in tissue culture in the presence of complement¹⁶ but infusion of high titer rheumatoid serum or white blood cells into normal volunteers was without harmful effect (fortunately!¹⁷). Also, numerous cases of rheumatoid arthritis have occurred in patients with agammaglobulinemia¹⁸ and in whom rheumatoid factor activity was lacking.¹⁹ It is apparent that attaching *in vivo* significance to *in vitro* cytotoxic reactions is precarious. Nevertheless, such studies may in time furnish important information regarding mechanisms of cell damage. Before considering cytotoxic reactions in specific diseases, we should turn our attention to certain aspects of behavior of cells in tissue culture, heterogeneity of immune globulins and basic aspects of the cytotoxic reaction.

Behavior of Mammalian Cells in Tissue Culture: Another problem is that of the properties of cells in tissue culture in relation to their behavior *in vivo*. Since the advent of antimicrobial agents tissue culture methods have become generally available and the nutritional requirements and behavior of cells from various animal tissues have been studied in great detail²⁰⁻²⁶. Among the

characteristics studied are cell morphology, biochemical behavior, tumor inducing capacity, chromosomal composition, viral susceptibility, drug sensitivity, antibody production, special functions such as hormone production and rhythmic contraction, and antigenic composition²⁷⁻³³. Briefly, many cells undergo a variety of changes in tissue culture, including changes in enzymatic activity, malignant transformation, and altered tumorigenicity, thereby adding another variable to experimental systems utilizing these methods. Some of the problems arising in isolated cell cultures ("tissue culture") may be circumvented by organ culture³⁴, in which a portion of an intact organ is maintained *in vitro* in artificial medium. However organ culture has not yet been widely applied to the problem under discussion.

One obvious question arises, and this is what happens to the immunologic reactivity of cells grown in tissue culture? If cells rapidly lose their antigenic constitution in culture this would compound the difficulties in interpretation of observations on immune cytotoxicity *in vitro*. In general, primary cell isolates and early subcultures of these cells seem to retain a number of antigens. In one study^{35,36} the H-2 isoantigen* was detected in continuously cultured mouse lymphoma cells six years after their establishment in tissue culture and in mouse fibroblasts cultured for one year. Other studies³⁷⁻⁴¹ have shown persistence of blood group antigens and transplantation antigens for varying periods of time, while kidney specific antigen was lost quite soon in cultured cells⁴².

So far as antibody formation by cultured cells is concerned, there is a much greater body of information⁴⁶. Antibody production *in vitro* has been demonstrated repeatedly, notably by cultured spleen, lymph node, and thymus cells. There are quantitative differences in rates of antibody formation when compared with the intact organism, although antibody formed *in vitro* has similar properties to that formed *in vivo* (chromatographic behavior, precipitating properties in the

*The H-2 isoantigen is one of several transplantation antigens of the mouse. It is involved in the homograft reaction, leukoagglutinin and hemagglutinin formation, and in the production of cytotoxic antibodies (36, 43-45).

presence of specific antigen, immuno-fluorescent reactivity, hemagglutination, susceptibility to mercaptoethanol* and the incorporation of radioactive amino acids⁴⁶⁻⁵⁰.

Heterogeneity of Antibodies: Brief mention should be made of the fact that humoral antibodies have certain differences aside from specific reactivity with given antigens. Of these, one of the better known is difference in molecular size. For example, most rheumatoid factors are 19 S gamma-1 microglobulins with a molecular weight of about 1 million and belong to the same class of antibody proteins as antipneumococcal antibody, A and B isohemagglutinins, typhoid O agglutinins, Wasserman antibody and cold agglutinins.⁵¹ Other antibodies such as most bacterial antibodies, fungal antibodies, viral antibodies, L-E cell factors, antinuclear factors, and the thyroid cytotoxic antibody are usually 7 S, gamma-2 globulins with a molecular weight of about 160,000. Some of these, including rheumatoid factor, antinuclear factor, antibodies to typhoid H antigen, and Wasserman antibody, are distributed in both 7 S and 19 S fractions in different individuals.^{52,53} Finally, the antibody formed early in a primary response is 19 S, while later it is 7 S. During the secondary response to a booster dose of antigen 7 S antibody predominates.⁵³⁻⁵⁸ One of the most notable areas in which heterogeneity of gamma globulins has been a problem is that of myeloma proteins, which differ antigenically as well as in their n-terminal amino acid composition. This has been reviewed in detail by Fahey⁵² and Putnam.⁵⁹

In summarizing, we might say: 1) Humoral cytotoxic antibodies have been demonstrated *in vitro* in certain disease states and experimental situations. (2) They are of interest because of their possible bearing upon mechanisms of cell damage in homograft reactions and autoimmune disease in man. (3) Cells in tissue culture undergo a number of changes but seem to retain certain antigenic traits and the ability to produce antibody for varying periods. (4) Hu-

moral or circulating antibodies belong to at least two major and different physical classes of protein and (5) These variables and others must be considered in evaluating and interpreting results from *in vitro* experiments.

We can now turn our attention to the test system involved; the events occurring during the cytolytic reaction, the role of antibody and complement in the reaction, and finally to the occurrence and significance of circulating cytotoxic antibodies in certain diseases.

The In Vitro Test System for Cytotoxic Antibody: Immune serum in the presence of complement is incubated with cellular antigen in tissue culture (guinea pig serum is employed as the complement source). The cells are then observed microscopically at varying intervals for cytolysis or they are examined for viability by exclusion of trypan blue.⁴ This effect may be observed as early as six minutes after addition of antibody and complement.⁶⁰ A number of other methods have been used to assay cytotoxic effect, including changes in motility, reduction in number of viable cells as measured by electronic counting, inhibition of metabolic processes such as incorporation of glycine, acid production, interference with cell division and inhibition of virus multiplication as assayed by plaque reduction.^{36,62-64,75} One other method has recently been employed, and this is the measurement of release of isotope from control as compared with damaged cells, employing p³²-orthophosphate, C¹⁴-amino acids, or C-¹⁴-thymidine.^{72,73}

In most of the experiments to date, mass culture of cells has been employed. Cells are seeded and incubated with medium containing varying amounts of antiserum and complement.^{16,36,60,74,75} Cytotoxic effect is then assayed by one of the previously mentioned methods. No effect is observed in the absence of complement. As previously mentioned guinea pig serum is the usual source of this reagent, but sometimes is unsuitable. For example, guinea pig serum is toxic without added antibody to mouse thymic

*Mercaptoethanol is an agent that causes the reduction of disulfide bonds, which are integral components of 19 S antibody proteins. Heterophil antibodies, cold agglutinins, 19 S human gamma globulin and 19 S rheumatoid factor have been treated with this reagent, with a resultant decrease in sedimentation velocity as determined in the ultracentrifuge and loss of biologic activity (e.g. sheep cell and latex agglutination in the case of rheumatoid factor). 68-71

lymphocytes, whereas hamster serum which has been absorbed with mouse red cells is a suitable source of complement.⁷⁶ Calcium and magnesium ions are also apparently essential for cytotoxicity, as judged by experiments employing disodium ethylenediaminetetraacetate,^{75,77} in which the chelating agent destroyed the cytotoxic effect of antiserum. This was reversible by repletion of calcium and magnesium ions in the test antiserum.

An exception to the requirement for complement has been described by Oda and Puck⁷⁸ in cloning experiments. They found that single cells may be killed without complement when antibody concentration exceeded 1 to 2 per cent, and that cells incubated in the presence of small amounts of antibody alone lose their sensitivity unless complement is added within 60 minutes. This again demonstrates the importance of the experimental system used in interpreting results of cytotoxicity studies.

The Role of Antibody and Complement: This aspect of cytotoxic antibody reactions has recently been reviewed by Bitensky.⁴ Antibody in the absence of complement seems to be limited to the cell surface as revealed by studies with fluorescein labelled antibody or ferritin conjugated antibody visualized with the electron microscope. However, intracellular lysosomes are also affected with consequent leakage of hydrolytic enzymes, and in the presence of complement, antibody is apparently more firmly fixed to the cell surface. It has been postulated that complement may act as an esterase affecting some component of the cell membrane thus permitting free penetration of antibody. Then disruption of lysosomes by antibody or antibody plus complement results in cell lysis by the released hydrolytic enzymes.^{4,79,120} This is an attractive notion but remains to be proved. The significance of the complement system is comprehensively reviewed by Osler,⁸² including variations of serum complement levels in a variety of autoimmune diseases.

The Relationship of Cytotoxic to

Other Humoral Antibodies: Most studies of humoral antibodies produced by tumor grafts or homografts have employed hemagglutination as an estimate of antibody response.^{6,85,86} In a series of absorption experiments, Jensen and Stetson⁸⁷ were unable to separate cytotoxic and hemagglutinating properties of hyperimmune sera, and they also observed that cytotoxic and hemagglutinating activities of these sera ran in parallel fashion. Correlations between other types of antibody and cytotoxic antibody have been observed in studies of thyroid antibodies, in which a close correlation between microsomal complement fixing antibody and cytotoxicity were observed.⁸⁸ As will be discussed in the following section, there seems to be no correlation between antibrain complement fixing antibody and cytotoxic demyelinating antibody.⁸⁹

OBSERVATIONS IN EXPERIMENTAL AND HUMAN AUTOIMMUNE DISEASE

Experimental Allergic Encephalomyelitis: In rabbits, the injection of heterologous central nervous system tissue in the presence of Freund's adjuvant, regularly results in progressive paraplegia and demyelination, the condition known as experimental allergic encephalomyelitis (EAE). The serum of these animals contains a gamma-2 globulin which is cytotoxic to tissue cultures of rat and mouse cerebellum in the presence of complement. Fluorescent labelled EAE serum is localized on the myelin sheath. The normal cytotoxic effect is eliminated by absorption with normal brain tissue,⁹² and is reversible by removal of the EAE serum from the culture. As previously mentioned, these sera show complement fixing antirat brain antibody,^{89,93} and this is apparently different from the cytotoxic factor. EAE can be prevented by injection of antiserum containing high titers of complement fixing antibody to the particular brain antigen.⁹⁶ The disease may be transmitted by sensitized lymphoid cells but not by hyperimmune serum.⁹⁵

In a concentration of 50 per cent, serum from 14 of 20 patients with multiple sclerosis in exacerbation produced a similar effect, while none of 15 sera from patients in remission were cytotoxic. One of 16 control sera and none of 18 sera from patients with other central nervous system disease were cytotoxic.⁹⁴ The question of the significance of humoral factors in the pathogenesis of multiple sclerosis, which these observations raise, is so far unanswered.

The Cytotoxic Factor in Thyroid Disease: A large proportion of sera from patients with Hashimoto's thyroiditis are cytotoxic to culture thyroid cells.⁸⁸ Again, this system is complement dependent. Patients with this disorder showed an incidence of 19% positive sera when tested for cytotoxicity. Sera from 9 patients with myxedema, from 34 of 51 patients with thyrotoxicosis and from 8 of 91 controls gave positive results. Of 4 patients with lupus erythematosus and no thyroid disease none was positive, while 2 of three SLE patients with thyroid disease were positive. The cytotoxic factor was shown to be a 7S gamma-2 globulin by zone density gradient ultracentrifugation, mercaptoethanol treatment, column chromatography and zone electrophoresis.^{88,97,98} Absorption studies showed loss of activity with use of thyrotoxic gland homogenate or microsomes, but not with mitochondria from the same gland.⁸⁸ Thyroid tissue from glands with colloid goiter was not effective.

Ulcerative Colitis: The presence of antibodies to colonic tissue in the sera of patients with ulcerative colitis has been firmly established by gel diffusion, hemagglutination, complement fixation, and fluorescent antibody methods.^{73,99,100-102} In one study⁹⁹ labelled sera from patients with rheumatoid arthritis also stained colon cells, but the pattern of staining was different from that observed with ulcerative colitis sera. In tissue culture studies¹⁰⁰ cytotoxicity, as estimated by morphology or release of p^{32} -orthophosphate from labelled colon cells, could not be demonstrated with ulcerative co-

litis sera in the presence of complement. Fluorescent staining and hemagglutinating activities of the sera were well correlated.

Following the suggestion from other transfer experiments,¹⁰³⁻¹⁰⁷ the previously mentioned investigators¹⁰⁰ studied the effect of leukocytes from patients with ulcerative colitis on the release of p^{32} -orthophosphate from colon cells. In this instance a significant increase in rate of isotope release was noted with ulcerative colitis leukocytes when compared with control leukocytes. Attempts to sensitize normal leukocytes by incubation with ulcerative colitis serum were unsuccessful. The presence of complement was required for the cytotoxic effect (increased p^{32} release) of ulcerative colitis leukocytes.

Disseminated Lupus Erythematosus: Cytotoxic antibodies have not yet been reported in sera of patients with this disorder, with the exception of two individuals with SLE and thyroid disease showing thyroid cytotoxic antibody.⁸⁸

Scheffer¹⁰⁸ found that sera from patients with SLE had no effect on chicken heart or human spleen cells in tissue culture so far as growth or general appearance were concerned. However, he did note the presence of "purple bodies" which resembled the hemotoxylin bodies seen in SLE. Serum from patients with rheumatoid arthritis did not produce this change. However, no experiments were performed with complement added to the system.¹⁰⁹

Rheumatoid Arthritis: Tissue culture of synovial cells has been carried out in several laboratories and to date no differences have been observed in the morphology or staining characteristics of rheumatoid and non-rheumatoid synovial cells.^{16,31,110-114}

Cytotoxic effects of rheumatoid sera in the presence of complement have been demonstrated in at least two laboratories.^{16,115} The usual concentration of rheumatoid serum in the medium is 40 to 50 per cent. No effect of rheumatoid serum plus complement was noted upon HeLa cells or monkey kidney cells.

The exact significance of rheumatoid factor is not settled. Most workers feel that it is an antibody directed against gamma globulin or antigen-antibody complexes,^{117,118} and that it is a result of, rather than a cause of the disease process. Also, Kaplan and Jandl¹¹⁹ have found that rheumatoid macroglobulins would agglutinate Rh-sensitized red cells *in vitro*, but that they would not accelerate their destruction *in vivo*. They suggest that native 7 S gamma globulins in normal serum inhibit rheumatoid factor activity in the blood stream, and that if rheumatoid factor is of pathogenetic significance it must act in an area of relatively low protein content, such as the synovial fluid.

DISCUSSION

With the data available at present, no definite role of humoral cytotoxic anti-

body can be established in homograft injection or in autoimmune disease in man. Immune serum cannot transfer disease, but sensitized cells can. Nevertheless, as Stetson and Paterson^{9,89} have pointed out, these experiments do not exclude cytotoxic antibody as a cause of cell damage. Local cellular release of humoral cytotoxic antibody in sufficient concentration to be effective is a definite possibility and the failure of immune serum to successfully transfer sensitivity may be, at least in part, quantitative. Indeed, this may partly explain the necessity for using such large amounts of immune serum to demonstrate cytotoxicity in the instances of EAE and rheumatoid arthritis. Chances are that this argument will be resolved someplace in "middle ground," just as happened in the argument over the mechanism of action of parathormone.¹¹⁶

SUMMARY

Among the various abnormal serologic reactions present in human and experimental autoimmune disease, cytotoxicity in the presence of complement is one of the more puzzling.

Antibody and complement are both required for cytotoxicity in most experiments reported to date. Their mechanism of action may involve enzymatic action on the cell membrane as well as release of lysozomal hydrolytic enzymes, although this remains to be proved.

In experimental autoimmune encephalomyelitis, Hashimoto's thyroiditis and rheumatoid arthritis cytotoxic antibodies

have been clearly demonstrated in serum. However, in ulcerative colitis, only sensitized leukocytes from patients will cause cytotoxicity of colon tissue cultures in the presence of complement.

Whether humoral cytotoxic antibodies cause tissue damage in naturally occurring autoimmune disease remains debatable. However, sensitized cells can continue to produce antibody *in vitro*, and local production of high concentration of cytotoxic antibody by sensitized cells may conceivably contribute to tissue destruction in these diseases.

REFERENCES

1. Mackay, I. R., and Burnet, F. M., *Autoimmune Diseases*, Springfield, Charles C. Thomas, 1963.
2. Gajdusek, D. C.: An "Autoimmune" Reaction Against Human Tissue Antigens in Certain Acute and Chronic Diseases. I. Serologic Investigations, *Arch. Int. Med.*, 101:30, 1958.
3. Donich, D., and Roitt, I. M.: Autoantibodies in Disease, *Ann. Rev. Med.*, 13:213, 1962.
4. Bitensky, L.: Cytotoxic Action of Antibodies, *Brit. Med. Bull.*, 19:241, 1963.
5. Green, H., and Goldberg, B.: The Action of Antibody and Complement on Mammalian Cells,

Ann. N.Y. Acad. Sci., 87:352, 1960.

6. Hasek, M., Lengerova, A., and Hrabá, T.: Transplantation Immunity and Tolerance, Adv. Immunol., 1:1, 1961.

7. Möller, G. and Möller, E.: Studies *in vitro* and *in vivo* of the Cytotoxic and Enhancing Effect of Humoral Isoantibodies, Ann. N.Y. Acad. Sci., 99:504, 1962.

8. a. Medawar, P. B.: Reactions to Homologous Tissue Antigens in Relation to Hypersensitivity, In Lawrence, H.S. ed.: Cellular and Humoral Aspects of Hypersensitive States, New York, Paul Hoeber, Inc., 1959, p. 504.

b. Billingham, R. E., Brent, L., Brown, J. B. and Medawar, P. B.: Time of Onset and Duration of Transplantation Immunity, Transplant. Bull., 6:410, 1959.

9. Stetson, C. A.: The Role of Humoral Antibody in the Homograft Reaction, Adv. Immunol., 3:97, 1963.

10. a. Hauschka, T. S.: Immunologic Aspects of Cancer, Cancer Res., 12:615, 1952.

b. Wissler, R. W.: Effects of Specific Antibodies on Tissue Cells, Ann. Rev. Microbiol., 16:265, 1962.

11. Imagawa, D. T., Syverton, J. T., and Bittner, J. J.: The Cytotoxicity of Serum for Mouse Mammary Cancer Cells. I. The Effects of Admixture *in vitro* upon Homiotransplantability, Cancer Res., 14:1, 1954.

12. Lambert, R. A. and Hanes, F. M.: The Cultivation of Tissues *in vitro* As a Method For the Study of Cytotoxins, J. Exper. Med., 14:453, 1911.

13. Goldstein, M. N., Hiramoto, R., and Pressman, D.: Comparative Fluorescein Labeling and Cytotoxicity Studies with Human Cell Strains HeLa, Rous, and 407-liver and with Fresh Surgical Specimens of Cervical Carcinoma, Osteogenic Sarcoma and Normal Adult Liver, J. Nat'l Cancer Inst., 22:697, 1959.

14. Goldstein, M. N.: The Cross Reactions of Antisera Prepared Against Four Human Cell Strains in Continuous Tissue Culture, J. Immunol., 79:113, 1957.

15. Kaliss, N.: Immunologic Enhancement of Tumor Homografts in Mice, Cancer Res., 18:992, 1958.

16. Fraser, J. R. E., and Catt, K. J.: Human Synovial Cell Tissue Culture. Use of a New Method in a Study of Rheumatoid Arthritis, Lancet, 2:1437, 1961.

17. Harris, J., and Vaughan, J. H.: Transfusion Studies in Rheumatoid Arthritis, Arth. & Rheum., 4:47, 1961.

18. Good, R. A., Rotstein, J., and Mozzitello, W. F.: The Simultaneous Occurrence of Rheumatoid Arthritis and Agammaglobulinemia, J. Lab. & Clin. Med., 49:343, 1957.

19. Vaughan, J. H., and Good, R. A.: Relation of Agammaglobulinemia Sera to Rheumatoid Agglutination Reactions, Arth. & Rheum., 1:99, 1958.

20. Eagle, H.: Metabolic Studies with Normal and Malignant Cells in Tissue Culture, Harvey Lectures, 54:156, 1960.

21. Puck, T. T.: Quantitation of Growth of Mammalian Cells, Harvey Lectures, 55:1, 1961.

22. Paul, J.: Cell and Tissue Cultures, Williams and Wilkins, Baltimore, 1960.

23. Henle, G. and Deinhardt, F.: The Establishment of Strains of Human Cells in Tissue Culture, J. Immunol., 79:54, 1957.

24. Tijo, J. H. and Puck, T. T.: Genetics of Mammalian Cells. II. Chromosomal Constitution of Cells in Tissue Culture, J. Exper. Med., 108:259, 1958.

25. Eagle, H., Barban, S., Levy, M., and Schulze, H. O.: The Utilization of Carbohydrates by Human Cell Cultures. J. Biol. Chem., 233:551, 1958.

26. Eagle, H.: Amino Acid Requirements in Mammalian Cell Cultures, Science, 130:432, 1959.

27. Thorbecke, G. J.: Gamma Globulin and Antibody Formation *in vitro*. I. Gamma globulin Formation in Tissues from Immature and Normal Adult Rabbits, J. Exper. Med., 112:279, 1960.

28. Pomerat, C. M.: Rhythmic Contractions of Schwann Cells, Science, 130:1759, 1959.

29. Levan, A., and Bieseke, J. J.: Role of Chromosomes in Carcinogenesis as Studies in Serial Tissue Culture of Mammalian Cells, Ann. N. Y. Acad. Sci., 71:1022, 1958.

30. McAllister, R. M., and Coriell, L. L.: Tumorigenicity of Tissue Culture Cells, Cancer Res., 19:1040, 1959.

31. Castor, C. W., and Fries, F. F.: Composition and Function of Human Synovial Connective Tissue Cells Measured *in vitro*, J. Lab. and Clin. Med., 57:394, 1961.

32. Chessin, L. N., and Hirschhorn, K.: Virus Resistance and Sensitivity in Cultured Human Synovial Cells as a Possible Genetic Marker, Exp. Cell. Res., 23:138, 1961.

33. Hull, R. N., and Tritch, O. J.: Characterization of Cell Strains by Viral Susceptibility, Nat'l Cancer Inst. Mono. No. 7, 161, 1962.

34. Gaillard, P. J.: Studies of Function in Organ Culture: Summary, Correlation, and Speculation, Nat'l Canc. Inst. Mono. No. 11, 143, 1963.

35. Cann, H. M., and Herzenberg, L. A.: *In vitro* Studies of Mammalian Somatic Cell Variation. I. Detection of H-2 Phenotype in Cultured Cell Lines, J. Exper. Med., 117:259, 1963.

36. Cann, H. M., and Herzenberg, L. A.: *In vitro* Studies of Mammalian Somatic Cell Variation. II. Isoimmune Cytotoxicity with a Cultured Mouse Lymphoma and Selection of Resistant Variants, J. Exper. Med., 117:267, 1963.

37. Defendi, V., Billingham, R. E., Silvers, W. K., and Moorhead, P.: Immunological and Karyological Criteria for Identification of Cell Lines, J. Nat'l Cancer Inst., 25:359, 1960.

38. Coriell, L. L., Tall, M., and Gaskell, H.: Common Antigens in Tissue Culture Cell Lines, Science, 128:198, 1958.

39. Hogman, C. F.: Blood Group Antigens A and B Determined by Means of Mixed Agglutination on Cultured Cells of Human Fetal Kidney, Liver, Spleen, Lung, Heart, and skin, Vox Sang., 4:319, 1959.

40. Brand, K. G., and Syverton, J. I.: Immunology of Cultivated Mammalian Cells. I. Species Specificity Determined by Hemagglutination, J. Nat'l Cancer Inst., 24:1007, 1960.

41. Kelus, A., Gurner, B. W., and Coombs, R. A.: Blood Group Antigens on Hela Cells Shown by Mixed Agglutination, *Immunol.*, 2:262, 1959.
42. Weiler, E.: Antigenic Comparison of Normal and Neoplastic Tissues, *Acta Univ. Internat. Contra. Cancrum.*, 15:929, 1959.
43. Pizarro, O., Hoecker, G., Rubenstein, P., and Ramos, A.: The Distribution in the Tissues and the Development of H-2 Antigens of the Mouse, *Proc. Nat. Acad. Sci.*, 47:1900, 1961.
44. Gorer, P. A.: The Antigenic Basis of Tumor Transplantation, *J. Path. and Bact.*, 47:231, 1938.
45. Gorer, P. A. and O'Gorman, P.: The Cytotoxic Activities of Isoantibodies in Mice, *Transplant. Bull.*, 3:142, 1956.
46. Stavitsky, A. B.: *In vitro* Studies of the Antibody Response, *Adv. Immunol.*, 1:211, 1961.
47. Askonas, B., and White, R. G.: Sites of Antibody Production in the Guinea Pig. The Relation Between *in vitro* Synthesis of Anti-Ovalbumin and Gamma Globulin and Distribution of Antibody Containing Plasma Cells, *Brit. J. Exp. Path.*, 37:61, 1956.
48. Patterson, R., Suszko, I. M., and Pruzansky, J. J.: *In vitro* Production of Antibody by Chicken Spleen Cells. I. Detection of Antibody by Isotope Techniques, *J. Immunol.*, 90:829, 1963.
49. Patterson, R., and Suszko, I. M.: *In vitro* Production of Antibody by Chicken Spleen Cells. II. Immunofluorescent Studies of Tissue Culture Preparations, *J. Immunol.*, 90:836, 1963.
50. Langevoort, H. L., Asofsky, R. M., Jacobson, E. B., DeVries, T., and Thorbecke, G. J.: Gamma Globulin and Antibody Formation *in vitro*. II. Parallel Observations on Histologic Changes and on Antibody Formation in the White and Red Pulp of the Rabbit Spleen During the Primary Response, with Special Reference to the Effect of Endotoxin, *J. Immunol.*, 90:60, 1963.
51. Kunkel, H. G.: Macroglobulins and High Molecular Weight Antibodies in Vol. I. The Plasma Proteins, ed. Putnam, F. W., New York, Academic Press, 1960, p. 279.
52. Fahey, J. L.: Heterogeneity of Gamma Globulins, *Adv. Immunol.*, 2:41, 1962.
53. Chodirker, W. B., and Tomasi, T. B.: Low Molecular Weight Rheumatoid Factor, *J. Clin. Invest.*, 42:876, 1963.
54. Spalluto, J., Miller, W., Dorward, B., and Fink, C. W.: The Formation of Macroglobulin Antibodies. I. Studies on Adult Humans, *J. Clin. Invest.*, 41:1415, 1962.
55. Bauer, D. C., and Stavitsky, A. B.: On the Different Molecular Forms of Antibody Synthesized by Rabbits During the Early Response to a Single Injection of Protein and Cellular Antigens, *Proc. Nat'l Acad. Sci.*, 47:1667, 1961.
56. Uhr, J. W., and Finklestein, M. S.: Antibody Formation. IV. Formation of Rapidly and Slowly Sedimenting Antibodies and Immunological Memory to Bacteriophage IX. 174, *J. Exper. Med.*, 117:457, 1963.
57. Stelos, P., and Taliaferro, W. H.: Comparative Study of Rabbit Hemolysins to Various Antigens. II. Hemolysins to the Forssman Antigen of Guinea Pig Kidney, Human Type A Red Cells, and Sheep Red Cells, *J. Infect. Dis.*, 104:105, 1959.
58. Benedict, A. A., Brown, R. J., and Ayenger, R.: Physical Properties of Antibody to Bovine Serum Albumin as Demonstrated by Hemagglutination, *J. Exper. Med.*, 115:195, 1962.
59. Putnam, F. W.: Aberrations of Protein Metabolism in Multiple Myeloma, *Physl. Rev.*, 37:512, 1957.
60. Hiramoto, R., Goldstein, M. N., and Pressman, D.: Limited Fixation of Antibody Viable Cells, *J. Nat'l Cancer Inst.*, 24:255, 1960.
61. Gorer, P. A. and O'Gorman, P.: The Cytotoxic Activity of Isoantibodies in Mice, *Transplant. Bull.*, 3:142, 1956.
62. Hirata, A. A.: Cytolytic Antibody Assay by Tryptic Digestion of Injured Cells and Electronic Counting, *J. Immunol.*, 91:625, 1963.
63. Terasaki, P. I., and Rich, N. E.: Quantitative Determination of Antibody and Complement Directed Against Lymphocytes, *J. Immunol.*, 92:128, 1964.
64. Roizman, B., and Roane, P. R.: Studies on Determinant Antigens of Viable Cells. I. A Method, and its Application in Tissue Culture Studies, for Enumeration of Killed Cells, Based on the Failure of Virus Multiplication Following Injury by Cytotoxic Antibody and Complement, *J. Immunol.*, 87:714, 1961.
65. Rhodes, A. J. and van Rooyen, C. E.: Textbook of Virology, 4th ed., Baltimore, Williams and Wilkins, 1962.
66. Hsiung, G. D. and Melnick, J. L.: Morphologic Characteristics of Plaques Produced on Monkey Kidney Monolayer Cultures by Enteric Virus (Poliomyelitis, Coxsackie and ECHO Groups), *J. Immunol.*, 78:128, 1957.
67. Itoh, H., and Melnick, J. L.: The Infection of Chimpanzees with ECHO Viruses., *J. Exper. Med.*, 106:677, 1957.
68. Deutsch, H. F. and Morton, J. I.: Dissociation of Human Serum Macroglobulins, *Science*, 125:600, 1957.
69. Grubb, R., and Swahn, B.: Destruction of Some Agglutinins but Not of Others by Two Sulfhydryl Compounds, *Acta Path. et Microbiol.*, 43:305, 1958.
70. Reisner, C. A., and Franklin, E. C.: Studies of Mercaptoethanol Dissociated Normal Human 19S Gamma Globulin and Pathologic Macroglobulins from Patients with Macroglobulinemia, *J. Immunol.*, 87:654, 1961.
71. Kunkel, H. G., Franklin, E. C., and Muller-Eberhard, H. J.: Studies on the Isolation and Characterization of the Rheumatoid Factor, *J. Clin. Invest.*, 38:424, 1959.
72. Klein, G., and Perlmann, P.: *In vitro* Cytotoxic Effect of Isoantibody Measured as Isotope Release from Labelled Target Cell DNA, *Nature*, 199:451, 1963.
73. Perlmann, P., and Broberger, O.: *In vitro* Studies of Ulcerative Colitis. II. Cytotoxic Action of White Blood Cells from Patients on Human Fetal Colon Cells, *J. Exper. Med.*, 117:717, 1963.
74. Oda, M., and Puck, T. T.: The Interaction of Mammalian Cells with Antibodies, *J. Exper. Med.*, 113:599, 1961.

75. Ross, A., and Lepow, I. H.: Studies on Immune Cellular Injury. I. Cytotoxic Effects of Antibody and Complement, *J. Exper. Med.*, 112:1085, 1960.
76. Reif, A. E.: Immune Cytolysis of Mouse Thymic Lymphocytes., *J. Immunol.*, 91:557, 1963.
77. Goldberg, B., and Green, H.: The Cytotoxic Action of Immune Gamma Globulin and Complement on Krebs Ascites Tumor Cells, *J. Exper. Med.*, 109:505, 1959.
78. Oda, M., and Puck, T. T.: The Interaction of Mammalian Cells with Antibodies, *J. Exper. Med.*, 113:599, 1961.
79. Dune, C. de: In: Hayashi, T., ed. *Subcellular Particles*, New York, Ronald Press, 1959, p. 128.
80. Green, H., Fleischer, R. A., Barrow, P., and Goldberg, B.: The Cytotoxic Action of Immune Gamma Globulin and Complement on Krebs Ascites Tumor Cells. II. Chemical Studies, *J. Exper. Med.*, 108:511, 1959.
81. Moeller, E., and Moeller, G.: Quantitative Studies of the Sensitivity of Normal and Neoplastic Mouse Cells to the Cytotoxic Action of Isoantibodies, *J. Exper. Med.*, 115:527, 1962.
82. Osler, A. G.: Functions of the Complement System, *Adv. Immunol.*, 1:131, 1961.
83. Ratnoff, O. D., and Lepow, I. H.: Complement as a Mediator of Inflammation, *J. Exper. Med.*, 118:681, 1963.
84. Haines, A. L., and Lepow, I. H.: Purification and Properties of Human C'I Esterase, *Fed. Proc.*, 21:17, 1961.
85. Gorer, P. A.: Antibody Response to Tumor Inoculation in Mice with Special Reference to Partial Antibodies, *Cancer Res.*, 7:634, 1947.
86. Medawar, P. B.: Immunity to Homologous Grafted skin. II. The Relationship Between the Antigen of Blood and Skin., *Brit. J. Exp. Path.*, 27:15, 1946.
87. Jensen, E., and Stetson, C. A.: Humoral Aspects of the Immune Response to Homografts. II. Relationship Between the Hemagglutinating and Cytotoxic Activities of Certain Isoimmune sera, *J. Exper. Med.*, 113:785, 1961.
88. Forbes, I. J., Roitt, I. M., Doniach, D., and Solomon, I. L.: The Thyroid Cytotoxic Autoantibody, *J. Clin. Invest.*, 41:996, 1962.
89. Paterson, P. Y.: Serum Factors in Experimental Allergic Encephalomyelitis and Neurologic Diseases of Man, *Nat'l Cancer Inst. Mono. No. 11*, 212, 1963.
90. Bornstein, M. B., and Murray, M. R.: Serial Observations on Patterns of Growth, Myelin Formation, Maintenance and Degeneration in Cultures of Newborn Rat and Kitten Cerebellum, *J. Biophys. and Biochem. Cytol.*, 4:499, 1958.
91. Bornstein, M. B. and Appel, S. H.: The Application of Tissue Culture to the Study of Experimental Allergic Encephalomyelitis, *J. Neuropath. and Exper. Neurol.*, 20:141, 1961.
92. Appel, S. H., and Bornstein, M. B.: The Application of Tissue Culture to the Study of Experimental Allergic Encephalomyelitis. II. Serum Factors Responsible for Demyelination, *J. Exper. Med.*, 119:303, 1964.
93. Paterson, P. Y.: Transfer of Allergic Encephalomyelitis in Rats by Means of Lymph Node Cells, *J. Exper. Med.*, 111:119, 1960.
94. Bornstein, M. B.: A Tissue Culture Approach to Demyelinative Disorders, *Nat. Cancer Inst. Mono. No. 11*, 197, 1963.
95. Chase, M. W.: A Critique of Attempts at Passive Transfer of Sensitivity to Nervous Tissue. In *Allergic Encephalomyelitis*, Kies, M. W. and Alvard, E. C., Jr., eds., Springfield, Charles C. Thomas, 1959, p. 348.
96. Paterson, P. Y., and Harwin, S. M.: Suppression of Allergic Encephalomyelitis by Means of Immune Serum, in Hasek, M., Lengerova, A., and Vojtiskova, M., eds., *Mechanisms of Immunological Tolerance*, New York, Academic Press, 1962, p. 507.
97. Irvine, W. J.: The Cytotoxic Factor in Thyroid Disease, *Scott. Med. J.*, 5:511, 1960.
98. Torrigiani, G., and Roitt, I. M.: Sedimentation Characteristics of Human Thyroid Autoantibodies, *Immunology*, 6:73, 1963.
99. Broberger, O., and Perlmann, P.: Demonstration of an Epithelial Antigen in Colon by Means of Fluorescent Antibodies from Children with Ulcerative Colitis, *J. Exper. Med.*, 115:13, 1962.
100. Broberger, O., and Perlmann, P.: *In vitro* studies of Ulcerative Colitis. I. Reactions of Patients' Serum with Human Fetal Colon Cells in Tissue Cultures, *J. Exper. Med.*, 117:705, 1963.
101. Asherson, G. L., and Broberger, O.: Incidence of Haemagglutinating and Complement Fixing Antibodies, *Brit. Med. J.*, 5237:1429, 1961.
102. Bregmon, E., and Kirsner, J. B.: Colon Antibodies in Ulcerative Colitis, *J. Lab. and Clin. Med.*, 56:795, 1960.
103. Paterson, P. Y.: Transfer of Allergic Encephalomyelitis in Rats by Means of Lymph Node Cells, *J. Exper. Med.*, 111:119, 1960.
104. Astrom, K. E., and Waksman, B. H.: Transfer of Allergic Encephalomyelitis in Rabbits by Means of Lymph Node Cells from Immunized Donor Rabbits, *J. Path. and Bact.*, 83-89, 1962.
105. Hess, E. V., Ashworth, C. T., and Ziff, M.: Transfer of an Autoimmune Nephrosis in the Rat by Means of Lymph Node Cells, *J. Exper. Med.*, 115:421, 1962.
106. Felix-Davies, D., and Waksman, B. H.: Passive Transfer of Experimental Immune Thyroiditis in the Guinea Pig, *Arth. and Rheum.*, 4:416, 1961.
107. Lawrence, H. S., *Delayed Hypersensitivity and the Behaviour of the Cellular Transfer System in Animal and Man*, in Shaffer, J. H., LoGrippe, G., and Chase, M. W., eds., *Mechanisms of Hypersensitivity*, Boston, Little, Brown and Co., 1959, p. 453.
108. Scheffer, E.: An Investigation into the Effect of Lupus Erythematosus (L.E.) Serum on Living Cells. The Finding of So-Called "Purple Bodies," *Koninkl. Nederl. Akad. Van Wetenschappen (Amsterdam)*, 64:501, 1961.
109. Scheffer, E.: Personal Communication, 1963.
110. Bartfeld, H.: Synovia of Rheumatoid and other Subjects: Growth Patterns: DNA and RNA Content—Studies with Fluorescent Fraction II., *Tissue Culture Assn. Abstracts*, Washington, D.C., May 29, 1962.

111. Stanfield, A. B., and Stephens, C.A.L.: Studies of Cells Cultured from 188 Rheumatoid and Non-Rheumatoid Synovial Tissues, *Tex. Rep. Biol. and Med.*, 21:400, 1963.
112. Chessin, L. N., and Hirschhorn, K.: Virus Resistance and Sensitivity in Cultured Human Synovial Cells as a Possible Genetic Marker, *Exptl. Cell Res.*, 23:138, 1961.
113. Vaubel, E.: The Form and Function of Synovial Cells in Tissue Culture. I. Morphology of the Cells under Varying Conditions, *J. Exper. Med.*, 58:63, 1933.
114. Castor, C.-W., Prince, R. K., and Dorstewitz, E. I.: Epithelial Transformation of Human Synovial Connective Tissue Cells: Cytologic and Biochemical Consequences, *Proc. Soc. Exp'tl Biol. and Med.*, 108:574, 1961.
115. Bartfeld, H.: Personal Communication, 1963.
116. Rasmussen, H.: Parathyroid Hormone, Nature and Mechanisms of Action, *Am. J. Med.*, 30:112, 1961.
117. a. Christian, C. L.: The Possible Significance of the Rheumatoid Factor, *Arth. and Rheum.*, 4:86, 1961.
117. b. Milgrom, F., and Witebsky, E.: The Rheumatoid Factor and its Possible Pathogenic Role, in Grabor, P., Miescher, P., eds., *Mechanisms of Cell and Tissue Damage Produced by Immune Reactions*, New York, Grune and Stratton, 1962, p. 337.
118. Vaughan, J. H. and Butler, V. P.: Current Status of the Rheumatoid Factor, *Ann. Int. Med.*, 56:1, 1962.
119. Kaplan, M. E., and Jandl, J. H.: The Effect of Rheumatoid Factors and of Antiglobulins on Immune Hemolysis *in vivo*, *J. Exper. Med.*, 117:105, 1963.
120. Weiss, L., and Dingle, J. T.: Lysosomal Activation in Relation to Connective Tissue Disease, *Ann. Rheum. Dis.*, 23:57, 1964.



THE EDITOR'S PAGE

The presentations in this second symposium and ninth issue of the Bulletin are in keeping with our stated intent . . . "to publish the results of the combined endeavors of this medical and scientific staff—the staff of a large metropolitan hospital, uniquely placed, dedicated to the care of people and engaged in teaching and research."

The papers in the current symposium deal with arthritis and related disorders. They represent the interest of certain members of our staff in arthritis, lupus erythematosus and immunology. In one instance combined experience with another hospital is presented dealing with some problems in the diagnosis of gout.

The topics chosen are believed to be worthy of emphasis because they illustrate an unusual aspect of a disease or because in the authors' experience fundamental considerations deserve reaffirmation. The individual papers come from five internists, two radiologists and one orthopedic surgeon. They emphasize the desirability of cooperation among various disciplines in the understanding, diagnosis and treatment of the arthritis patient and the patient afflicted with other forms of collagen disease.

Dr. Buenger and Dr. Bogdonoff describe respectively the value of arthrography in the study of knee joint pathology and illustrate the changes of temporomandibular joint disease in rheumatoid arthritis. Dr. Stuppy reviews the side effects of drugs used in the treatment of arthritis. Although the side effects of steroids are well known, occult gastrointestinal bleeding with salicylates and the ocular effects of chloroquine merit particular attention. Dr. Lowe reminds us with an unusual patient of the bone necrosis which may occur in collagen disease with or without benefit of corticosteroids. Dr. Hejna reiterates some of the principles and procedures of orthopedic surgery which may be helpful in improving comfort and function in the arthritic patient. Dr. Barton and his co-authors cite their experience in problems of making the correct diagnosis in gout. This is of importance because gout treated over the long term with drugs such as probenecid and sulfinpyrazone and now

*4-hydroxypyrazolo (3, 4-d) pyrimidine. This inhibits xanthine oxidase and consequently uric acid production ².

perhaps HPP* is the only form of progressive arthritis in which specific remedial therapeutic agents are available. Another problem facing the practicing physician is that of establishing with certainty whether the patient with active systemic lupus erythematosus has significant renal involvement. Dr. Pollak and his colleagues point out that renal biopsy is required, because none of the other clinical tests available at the present time will determine this. In conclusion, we examine one of the basic questions relating to the immunology of connective tissue disease, namely whether or not circulating antibodies have anything to do with the pathogenesis of tissue damage in these disorders.

Inevitably, we must turn to the question posed by Dr. Russell Cecil³ "How many rheumatoid patients are receiving first rate medical care?" We can extend this question to patients with other forms of chronic arthritis, and must conclude that it is not enough. One problem is the frustration to physicians of long term care of the patient with arthritis. But more important is the fact that we do not know the causes of the various forms of chronic arthritis. In the case of rheumatoid arthritis, virus or bacterial causation⁴ and "autoimmunity"⁵ have been incriminated but the question remains unanswered. Until these problems are solved we must do our best to diagnose and treat properly the arthritic with the tools we have.

ROBERT E. DEDMON, M.D., *Special Editor*
Symposium on
Arthritis and Related Disorders

REFERENCES

1. Gutman, A. B.: The search for uricosuric agents suited to the management of tophaceous gout, *New York State J. Med.*, 63:576, 1963.
2. Klinenberg, J. R., Goldfinger, S., Miller, J., and Seegmiller, J. E.: The effectiveness of a xanthine oxidase inhibitor in the treatment of gout, *Arth. & Rheum.*, 6:779, 1963.
3. Cecil, R. L.: How many rheumatoid patients are receiving first rate medical care?, *Arth. & Rheum.*, 1:185, 1958.
4. Ford, D. K.: Rheumatoid Arthritis—An infection?, *Arth. & Rheum.*, 6:159, 1963.
5. Mackay, I., and Burnet, F. M.: *Autoimmune Disease*, Springfield, Charles C. Thomas, 1963.



TABLE OF CONTENTS

<i>page</i>	
146.....	Reoviruses NEVILLE E. STANLEY, D.Sc.
151.....	Measurement of Pulse Rate at the Bedside RICHARD A. CARLETON, M.D., ALLEN F. BOWYER, M.D., AND JOHN S. GRAETTINGER, M.D.
155.....	Ultrasonic Aerosol Generator—A New Dimension In Aerosol Therapy ALBERT H. ANDREWS, JR., M.D.
162.....	Neoplasma of the Liver and Urinary Bladder in Rats Fed 2-Acetylaminofluorene and Indole: The Effect of Pre- vious Injections of Carbon Tetrachloride on the Neo- plasms RYOICHI OYASU, M.D., JAMES H. McDONALD, M.D. AND GEORGE M. HASS, M.D.
168.....	Anticoagulant Therapy: Theoretical and Practical Aspects- Review Article JOHN H. OLWIN, M.D. AND JOHANNES L. KOPPEL, Ph.D.
178.....	Abstracts and Reviews

REOVIRUSES

NEVILLE E. STANLEY, D.Sc.¹

There are many unanswered problems associated with reovirus infection. Why are they apparently the most ubiquitous of viruses yet described? What diseases do they cause in man and animals? Why is clinical evidence of infection confined to children? What are the hazards of neonatal infection? Is reovirus infection associated with tumor production? What is the nature of the relationship between human strains of reovirus and a large group of plant-insect viruses? Will reoviruses replicate in plant or insect cells?

Some of the observations briefly referred to in this report may throw some light on the problems and raise others. I would like to emphasize that all we are privileged to see is a brief glimpse of an evolutionary process in action. In presenting this glimpse I shall offer an historical approach and draw heavily on our Australian studies for this purpose.

It was in Sydney in 1951 that the first reovirus was isolated from the faeces of an aboriginal native child presenting with steatorrhoea and pneumonitis, and who later developed alopecia areata^{1,2}. The infective agent was characterized as a virus possessing hitherto undescribed properties. It was 70 m μ in diameter, resistant to many germicides and ether, not related to any known virus, and it produced an unusual clinical syndrome in newly born mice which were the only animals shown, at that time, to exhibit clinical evidence of infection. This syndrome, which could be regularly produced by oral or intranasal inoculation, or even by a few seconds contact with an infected mouse, comprised an oily hair effect, steatorrhoea, retardation of growth, jaundice, tremors and a characteristic mincing ataxic gait. The few mice that recovered from this acute illness usually developed as runts and frequently

showed alopecia in the lumbar and occipital regions, and a phase of secondary jaundice. The virus was named HEV (heptoencephalomyelitis virus) to describe the main lesions and symptoms of the disease in mice. Lesions were also seen in the heart, lungs and pancreas. Antibody estimations showed that a high percentage of humans in Australia had probably been infected with HEV. The next appearance of the virus was recorded in 1957 in a familial infection in Holland³ and the following year in a colony of mice in Poona, India (Work, 1958, personal communication). In 1959, viruses originally designated as ECHO type 10 were separated from the ECHO group and named "reovirus"* (Sabin, 1959). We soon realized that virologists in the U.S.A. who had been primarily working with tissue cultures and not infant mice, were in fact working with HEV⁵. There are now known to be three antigenic types of reovirus and HEV is reovirus type 3. All three types produce a haemagglutinin for human group O red cells, but only type 3 produces a haemagglutinin for bovine red

¹ Professor and Chairman Department of Microbiology, University of Western Australia, Perth, W. A., Australia.

Presented as a special lecture under the auspices of the Department of Microbiology, Friedrich Deinhardt, Chairman.

*Respiratory enteric orphan virus

cells. The latter haemagglutinin is unique in its action.⁷ HEV, or reovirus type 3, also differs from types 1 and 2 in other important properties.⁶

We now know (1) that the three types of reovirus are icosahedral RNA viruses with a protein coat comprised of 92 prismatic subunits, (2) that antibody to all three types of reovirus is widely distributed in nature, and (3) that the replication process and RNA of reovirus type 3 is so far unique among RNA animal viruses.

Australian studies have followed two directions—one epidemiological and the other clinical and pathological.

Australian Epidemiology Studies

Antibodies to the three types of reovirus have been found in many animals closely associated with man although the largest number of virus strains have been isolated from children and none from adults. Some years ago we instituted a study to test the hypothesis that man constituted a major reservoir of reovirus, and that animals coming into contact with man became infected and developed antibodies. This study was carried out with a rare Australian marsupial known as the Quokka (*Setonix brachyurus*), which



Fig. 1.—Australian Quokka (*Setonix brachyurus*)

has existed in many thousands on the small island of Rottnest, eleven miles off the coast at Perth, Western Australia. (Fig. 1 and 2).

The purpose in selecting the quokka was to determine whether quokkas living in close association with man possessed more antibodies to reovirus than those which had little or no association with man. The quokkas on Rottnest Island were thought to be ideal for this purpose because:

1. They do not migrate from one area to another but remain within a few hundred square yards of territory for years.

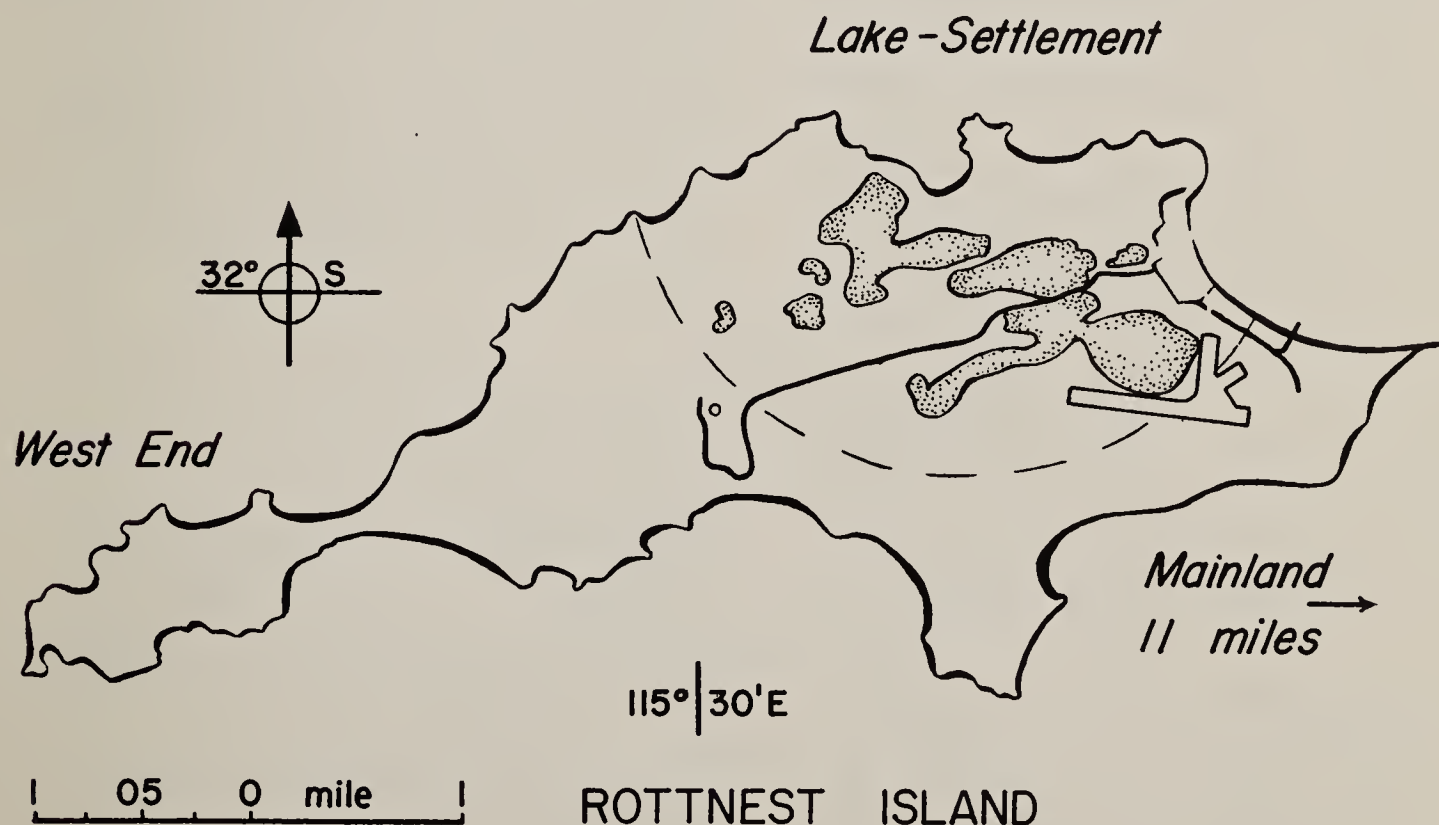


Fig. 2.—Rottnest Island

2. Quokkas on the West end of Rottnest Island are separated from the quokkas on the main part of the island, and have had very little contact with man compared with quokkas near the settlement.
3. Quokkas are nocturnal, vegetarians, are easily caught and bled by heart puncture, and have been assiduously studied by zoologists, physiologists and ecologists.

The results recorded in Table 1 lend support to the view that man could be a primary reservoir.¹⁰

However, we were not satisfied, and decided to examine the only other known source of quokkas in the world—those on a remote island off the Southern Coast of Australia. It is called Bald Island.

Because of its rugged nature, its remoteness, and the danger associated with attempts to land on this island, it was thought that the quokkas there would have had almost no contact with man. There are no records of landings by early explorers, and the predominant fauna are quokkas and lizards. A successful landing was made and specimens collected. The results were unexpected. Almost all the quokkas examined possessed both haemagglutination-inhibiting (HI) and virus neutralizing (VN) antibodies to the three types of reovirus¹⁰. In addition, reovirus type 3 was isolated from one quokka. How did the three types of reovirus become endemic in the quokkas of Bald Island?

Both HI and VN antibodies have now been demonstrated in a variety of Australian marsupials, including kangaroos, wallabies and quokkas. In addition, antibodies have been detected in all vertebrate sera tested except in those of sperm and hump-backed whales.⁸ Sera were collected from horses, sheep, cattle, fowls, monkeys, dogs, rats, mice, rabbits, bats and rainbow trout. If these are in fact antibodies present in the vertebrate sera, are they present as a result of a past infection with reovirus? Or are we dealing with a human virus, an animal virus, a plant virus, an insect virus, or a phenomenon of a bizarre sharing of antigenic components? Some light was thrown on the problem when Gomatos and Tamm¹¹ demonstrated that reovirus type 3 possessed an RNA that was double stranded. This is the only animal virus shown so far to have a double stranded RNA molecule. This unusual property is apparently shared by an insect transmitted plant virus, the wound-tumor virus (WTV), which replicates in the leaf-hoppers responsible for transmission. WTV is the same size as reovirus (70 mμ), has the same icosahedron shape with 92 protein subunits, and, like reovirus it replicates in the cytoplasm of the cell and not in the nucleus.¹² Rice-dwarf virus is the same size and shape as reovirus and replicates in the cytoplasm.¹⁴ This virus is also transmitted to plants by leaf-hoppers in which it multiplies. It was recently claimed (Streissle and Maramorosch, 1963) that wound-tumor

TABLE 1
Percentages of Quokkas with detectable H-I antibody (>1/10) against reovirus types 1, 2 and 3

Locality	No. of animals	Percentage positive against reovirus type			Contact with man
		1	2	3	
West End, Rottnest Island	35	17	34	28	rare
Lakes-Settlement, Rottnest Island	27	67	89	93	frequent
Mainland	10	70	100	100	continuous

virus (WTV) and reovirus share a common complement-fixing antigen. We have not been able to confirm this. So far we have been unsuccessful in attempting to cultivate reoviruses in plant cells or in cultures of blue-green algae, or WTV in mammalian cells. Tests on insect transmission of reovirus are being undertaken in view of our recent isolation of strains of reovirus type 3 from mosquitoes.¹⁷

Our epidemiological observations could possibly be explained then by one or more of at least four alternatives:

- a. Viruses designated as reovirus and known to replicate in animal cells may also be capable of replicating in either insect tissues, plant tissues, or in both: animals feeding on either the infected insects or plants, or bitten by the viruliferous insects, might then suffer apparent or inapparent infection and develop antibody;
- b. wound-tumor viruses may be able to replicate in animal cells: animals eating virus-infected plant cells would then become infected with wound-tumor virus and develop anti-body to an antigen shared with reovirus;
- c. the presence of antibody in animals may be caused by the ingestion of wound-tumor virus antigen in plant tissue: the animals could then develop antibody to the antigen without infection, but following absorption of reovirus antigens from the gastrointestinal tract;
- d. reoviruses and some plant cells may share common antigens.

As indicated earlier we may merely be seeing a few brief moments of an evolutionary process in which some members of a large group of plant-insect viruses have become adapted to the gastrointestinal tract of herbivores and then be spread either by faecal-oral contact or by insects to other vertebrates.

Australian Studies on Pathogenesis

It has been shown that oral infection of newly born mice with reovirus type 3

permits the establishment of a chronic disease which persists for many months and may terminate fatally^{9,10,17}. The clinical syndrome of the chronic disease is indistinguishable from the runting syndrome produced by the inoculation of immunologically competent lymphoid cells from an adult into a newly born mouse. This is generally considered to be a graft-versus host type of immunological reaction set up during the period of immunological tolerance.

An examination of the literature on runt disease reveals a clinical and pathological picture of great variability. A general pattern of agreement is emerging and it is apparent that variations may be ascribed to cell dosage, route of inoculation, time of inoculation, source of lymphoid cells, species of animal, and the genetic constitution of host and donor. In mice the following features have been stressed: retardation of growth, an ataxic gait, diarrhoea, hunched posture, facial edema, oily hair effect, jaundice, and alopecia. Up to 90 per cent of mice may show focal, necrotic, coagulative liver lesions. Some investigators report that the thymus may appear normal in size and appearance. Although hemolytic anemia with a positive Coombs' test, leucopenia and splenomegaly have frequently been observed, these are not an invariable accompaniment of runting.

The clinical syndrome of reovirus infection has only been demonstrated in infant mice less than five days of age and usually comprises retardation of growth, oily hair effect, diarrhoea, alopecia, jaundice, an ataxic mincing gait, and hunched posture. Although all mice show focal necrotic, coagulative liver lesions, the thymus appears normal. There is, also, following intraperitoneal inoculation, a peritoneal ascites containing large numbers of primitive lymphoid cells.

These similarities may be considered alongside the observations that (1) polyoma virus may cause runting and anemia; (2) mouse hepatitis viruses have been associated with runting; (3) cytomegalic inclusion disease virus infec-

tion of infants may result in sequelae which have been confused with erythroblastosis fetalis; (4) reovirus infections of humans appear to be mainly in infants; (5) the reovirus-induced lesions of liver or skeletal muscle closely resemble those seen in immunological injury of those same organs.

It would thus appear that further exploration of virus infection of the immunologically immature should be made to determine whether such infection will permit the establishment of a clone of cells which can react against the host.

In view of the observations that (1) reovirus replicates in close association with the mitotic apparatus of the cell cytoplasm (Spendlove et al., 1963), (2) reovirus may change cells so that they react against the host, and (3) reovirus type 3 appears to be in some way related to a virus producing tumours in plants,

it would seem worthwhile exploring the possibility that reovirus type 3 possesses some oncogenic potential. If indeed this should prove to be correct, great interest must surely be shown in the properties of a double-stranded RNA molecule in a highly ubiquitous infectious agent of man.

Our studies, so far, have served to highlight three aspects of infection and resistance. They are: 1. Investigation of neonatal virus infection should be considered in relation to disease developing later in life, or in maturity, with particular reference to autoimmune phenomena. 2. There would appear to be a need for greater integration between human, veterinary, plant and insect virologists, with regard to evolution, epidemiology, antigenicity, and cell susceptibility. 3. Reoviruses should be examined for oncogenic potential.

SUMMARY

Reoviruses are one of the most widely dispersed group of infective agents known in nature.

They are among the most highly infectious, durable and resistant viruses yet described.

They are associated with growth-interfering properties (runting).

Reoviruses are proving to be of value in unravelling problems of microbial evolution and epidemiology.



REFERENCES

1. Stanley, N. F., Dorman, D. C., and Ponsford, J.: *Aust. J. Exp. Biol. & Med. Sci.*, 31, 147, 1953.
2. Stanley, N. F., Dorman, D. C. and Ponsford, Jr.: *Ibid*, 32, 543, 1954.
3. van Tongeren, H. A. E.: *Archiv. of Virusforschung*, 7, 429, 1957.
4. Sabin, A. B.: *Science*, 130, 1387, 1959.
5. Stanley, N. F.: *Nature*, 189, 687, 1961.
6. Stanley, N. F.: *Med. J Aust.*, 2, 815, 1961.
7. Gomatos, P. J. and Tamm, I.: *Virology*, 17, 455, 1962.
8. Stanley, N. F. and Leak, P. J.: *Am. J. Hyg.*, 78, 82, 1963.
9. Stanley, N. F., Leak, P. J., Grieve, G. M. and Perret, D. H.: *Aust. J. Exp. Biol.*, 1964. (In press)
10. Stanley, N. F., Leak, P. J., Walters, M. N.-I. and Joske, R. A.: *Brit. J. Exp. Path.*, 1964. (In press)
11. Gomatos, P. J. and Tamm, I.: *Proc. Nat. Acad. Sci.*, 49, 707, 1963.
12. Bils, R. F. and Hall, C. E.: *Virology*, 17, 123, 1962.
13. Safferman, R. S. and Morris, M. E.: *Science*, 140, 679, 1963.
14. Fukushi, T., Shikata, E. and Kimura, I.: *Virology*, 18, 92, 1962.
15. Streissle, G. and Maramorosch, K.: *Science*, 140, 996, 1963.
16. Walters, M. N.-I., Joske, R. A., Leak, P. J. and Stanley, N. F.: *Brit. J. Expt. Path.*, 44, 427, 1963.
17. Stanley et al, unpublished observations.

MEASUREMENT OF PULSE RATE AT THE BEDSIDE

RICHARD A. CARLETON, M.D.,¹

ALLEN F. BOWYER, M.D.,²

JOHN S. GRAETTINGER, M.D.³

Assessment of pulse rate is probably performed more frequently than any other clinical measurement; however, despite the reliance often placed on this estimate of heart action, we are not aware of any studies which assess the accuracy of measurement of pulse rate.

The existence of patients who have had electronic pacemakers inserted for control of the ventricular rate has provided an opportunity to estimate the error in pulse rate measurements.

MATERIAL AND METHODS

The records of 5 patients during their postoperative periods form the basis of the study. Each patient had complete A-V block, which either had produced Stokes-Adams episodes (4 patients) or had been associated with the develop-

ment of congestive heart failure (1 patient). Each had had an implantable cardiac pacemaker* inserted during thoracotomy. The postoperative paced QRS rate was monitored on an oscilloscope initially but was also recorded electrocardiographically at least 3 times during the first 10 days postoperatively. The paced rate was measured from the electrocardiogram and was considered to represent the "true" heart rate.

The pulse rates counted at the cardiac apex or at a brachial or radial artery and then recorded in the clinical chart provide the estimates of pulse rate. These pulse measurements were made by registered nurses and by practical nurses, all of whom have been taught and requested to count the pulse for 1 full minute in patients with heart disease.

The differences between the "true" and the recorded heart rates have been tabulated and analyzed.

From the Section of Cardio-Respiratory Diseases, Department of Medicine, Presbyterian-St. Luke's Hospital, and the Department of Medicine, University of Illinois College of Medicine, Chicago, Illinois.

This work was supported in part by grants from the U.S. Public Health Service, National Institutes of Health (HE-05579), and the Chicago and Illinois Heart Associations.

¹ Assistant Attending Physician and Associate Director, Section of Cardio-Respiratory Diseases, Presbyterian-St. Luke's Hospital; Assistant Professor of Medicine, University of Illinois College of Medicine.

² National Heart Institute Postdoctoral Fellow in Cardio-Respiratory Diseases.

³ Attending Physician and Director, Section of Cardio-Respiratory Diseases, Presbyterian-St. Luke's Hospital; Associate Professor of Medicine, University of Illinois College of Medicine.

*Medtronics Corp., Minneapolis, Minn.

RESULTS

Early in the analysis, a remarkable tendency for the pulse rate to rise when the temperature rose (Fig. 1) suggested either an effect of temperature on the pacemaker or a “conditioned reflex” among the personnel. The former possibility was tested by immersing 3 pace- makers for 20 minutes at each of 3 tem- peratures in a water bath while record- ing the pacemaker rate on an oscillo- scope. The results of this inquiry were:

TABLE 1.
Effect of Temperature on
Pacemaker Rate

Temperature of Water Bath	Pacemaker Rate		
	Unit 1	Unit 2	Unit 3
22° C	78	69	49
35° C	79	72	53
41° C	79	73	54

Thus, variations of pacemaker rate oc- curring within the range of ordinary body temperatures are unlikely to exceed 1 impulse per minute.

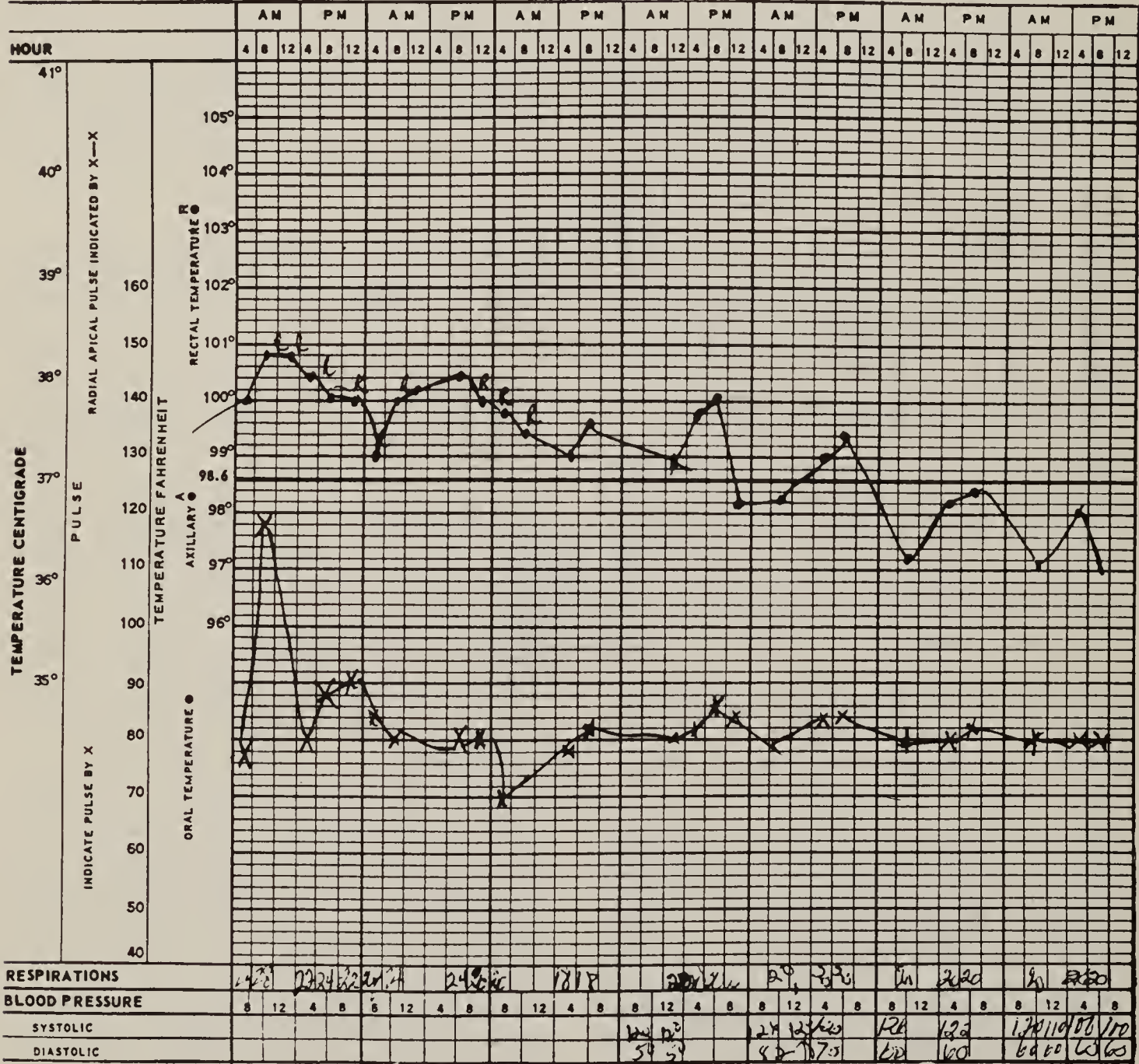


Fig. 1—Photograph of a 7-Day Segment of the Temperature-Pulse-Blood Pressure Chart from Patient L. G. Demonstrating the Apparent Tendency for the Recorded Pulse Rate and the Temperature to Change Together.

TABLE 2
Differences Between "True" Heart Rate and Pulse Rate

Patient	"True" Rate	Number of Observations	Mean Difference	Standard Error
A.H.	77	57	-1.18	0.49
B.G.	79	89	-0.82	0.49
L.G.	79	66	+1.18	0.43
F.W.	72	76	+2.46	0.59
H.S.	70	80	+3.21	0.61
Group of Five	—	368	+1.01	0.26

The differences between the "true" rates and pulse rates for the 5 patients individually and for the group of 5 are tabulated in Table 2. The frequency distribution of these differences is shown in Figure 2. Of the observations made, 38.3 per cent lie within ± 1 beat per minute of the "true" rate, and 73.1 per cent fall within ± 5 beats per minute. Two standard deviations encompass a range of ± 10 beats per minute. None of the individual mean pulse rates differ significantly from the "true" rate, but in all instances the observed discrepancies from the "true" rate are significant.

An ancillary observation was that only 3 of 368 pulse-rate measurements were odd numbers; the other 365 were even numbers.* The apparent conclusion from this unlikely event that heart rate changes only by jumps of 2 beats per minute (rarely being clocked during a transition) also seems unlikely in the face of the alternate possibility that pulse rate is counted for 15 or 30 seconds and multiplied by 4 or by 2.

COMMENT

The error of measuring heart rate by taking the pulse, defined as the range of 2 standard deviations, was ± 10 beats per minute in this study.

The reasons for this discrepancy are not entirely clear, but they warrant brief consideration. Had the pulse rates been exclusively, or even dominantly, less than the recorded "true" rate, one might

postulate pulse pressures of widely varying magnitude, some of which were below the threshold of palpating fingers. Such variation in pulse amplitude is well recognized with pacemakers in atrioventricular dissociation¹ and may have played a role in this study. Such an explanation cannot, however, explain pulse rates greater than the "true" rate. These "high" pulse rates could be due to an intrinsic pacemaker's outstripping the electronic pacemaker rate, either from a ventricular focus or by the mechanism of loss of the complete atrioventricular block with capture of the ventricles by an atrial pacemaker. The entire series of 18 electrocardiograms obtained during the series of pulse rate observations from all 5 patients were reviewed. Nine ventricular premature beats, 2 of which were interpolated, were recorded in a total of 19 minutes of electrocardiographic recording in these 18 tracings. Thus, even if each of these had produced a pulse of palpable amplitude and if each had been interpolated, ventricular premature beats are unlikely to have produced the excess of pulse rate over "true" rate which was found. Neither during the early post-operative days of continuous oscillographic monitoring nor on the recorded electrocardiogram was ectopic ventricular tachycardia seen. In no electrocardiogram obtained as long as 3 months post-operatively was atrioventricular conduction seen.

*The probability of obtaining 3 or less odd pulse rates (assuming that each count was taken for 1 minute) from a total of 368 pulse rate observations is:

$$p = \left(\frac{1}{2}\right)^{368} + 368 \left(\frac{1}{2}\right)^{368} + \frac{368 \times 367}{2} \left(\frac{1}{2}\right)^{368} + \frac{368 \times 367 \times 366}{6} \left(\frac{1}{2}\right)^{368}$$

or 1.4 chances in 10^{102} .

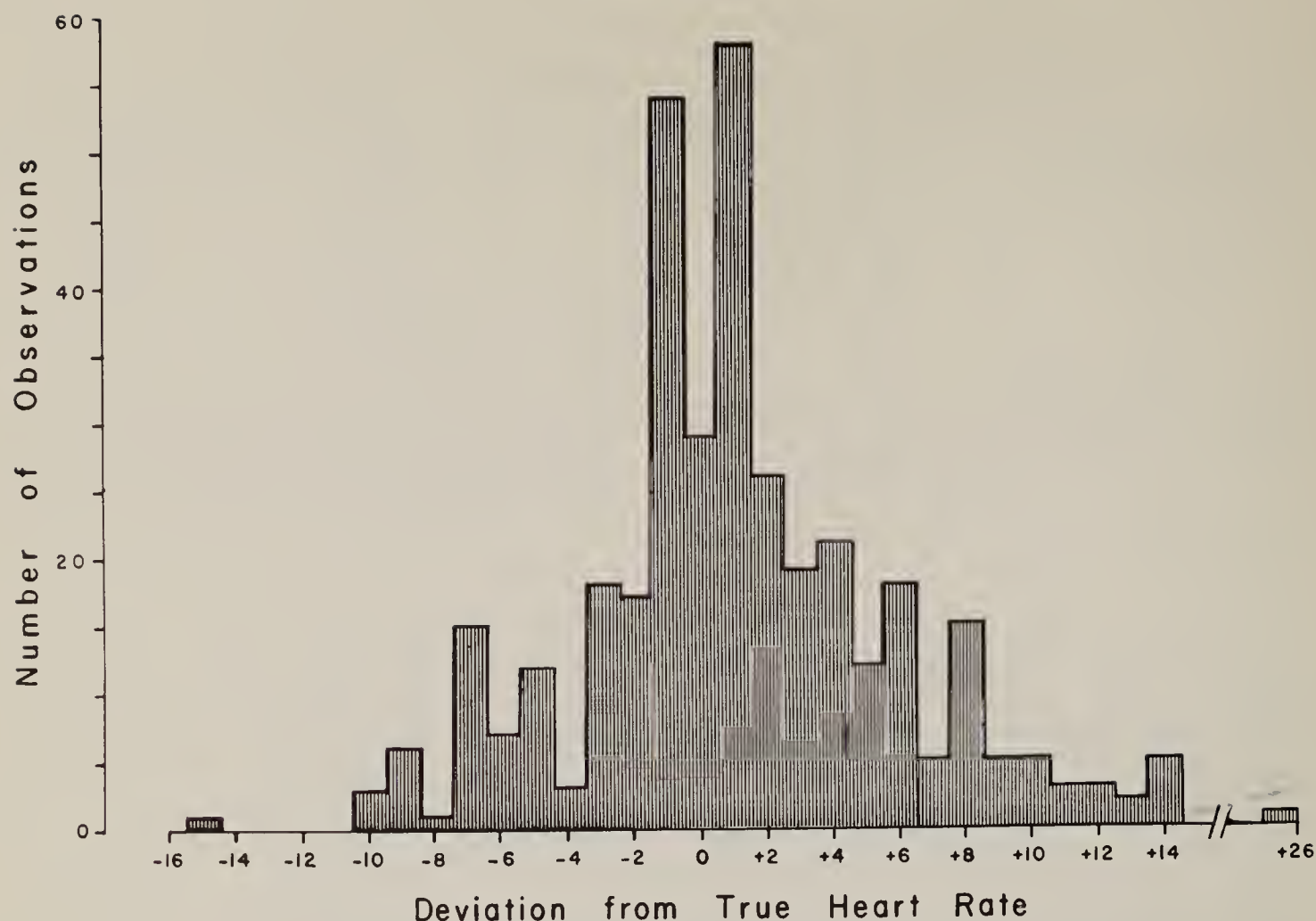


Fig. 2—The Frequency Distribution of "True" Heart Rate-Pulse Rate Differences in 368 Observations of Pulse Rate.

The lack of satisfactory cardiac explanations for the pulse rate discrepancies and the nearly random distribution of the deviations from the "true" rate lead to the conclusion that observer error is causal.

The magnitude of the error of pulse

rate measurement is definable; the magnitude of the clinical effect of such error is difficult to define. This study serves to point out the necessity for continuing awareness of the possible fallibility of man as a measuring and recording instrument.

ACKNOWLEDGEMENT

We are grateful to Mr. Mitchell Passavoy, Director of the Section of Biostatistics, Presbyterian-St. Luke's Hospital for his assistance with the probability calculations.

REFERENCE

1. Samet, P., Jacobs, W., Bernstein, W. H., and Shane, R. Hemodynamic sequelae of idioventricular pacemaking in complete heart block. *Am. J. Cardiol.* 11:594-599, 1963.

ULTRASONIC AEROSOL GENERATOR:

A new dimension in Aerosol Therapy;

Preliminary Report

ALBERT H. ANDREWS, JR., M.D.¹

Many diseases of the tracheobronchial tree and lungs cause inadequate elimination of secretions. Therapy begins with increased fluid intake, expectorants, postural drainage, proceeds to the application of water and saline aerosol, detergents and enzymes, cough machines, and finally to the aspiration of secretions and irrigation through a catheter, bronchoscope or tracheostomy. The application of fluid to the tracheobronchial tree has progressed with technical improvements. First used were steam inhalations, later room humidifiers, and most recently, aerosol generators.

Conventional generators utilize compressed gas, usually oxygen, and have been improved by increasing production of aerosol, and more efficient delivery to the patient. The production of an aerosol by the use of ultrasonic vibration makes it possible to deliver to the patient a much larger quantity of fluid than has heretofore been possible.

BACKGROUND

To be meaningful, a study of aerosol therapy must take into account the quantity of aerosol delivered to the patient. The estimation of aerosol output by measurement of water consumed is fundamental, but is time consuming. A recording optical densitometer was constructed to incorporate a light source, photosensitive transducer, and heated windows. The measurement of output by means of optical density has been shown to be both reliable and accurate (Fig. 1).

From the Department of Otolaryngology and Bronchoesphagology, Division of Surgery

¹ Attending Bronchoesphagologist Presbyterian-St. Luke's Hospital; Clinical Professor of Bronchoesphagology, Otolaryngology Department University of Illinois College of Medicine

Supported by a research grant from the DeVilbiss Company with contributing support from American Medical Association, American College of Chest Physicians and the Linde Company.

Maximum aerosol dosage is achieved by:

1. selection of appropriate make of generator
2. operation at high gas flow rate
3. use of air diluter, or modified reservoir-type mask
4. application of heat

The remarkable variation in the output of generators is seen in Figure 2 which presents graphically the aerosol output of generators of various manufacturers. On the other hand, the generators of a single manufacturer show remarkable uniformity of behavior (Fig. 3).

Variation of output with flow rate of the propellant gas is also seen in Figures 2 and 3.

Mechanisms for air dilution, incorporated in some generators, increase the quantity of aerosol delivered (Fig. 4 and 5). Such mechanisms permit air to enter the generator to meet peak inspiratory demand. The additional air is humidified chiefly by large particles of water within the generator. Without such mechanisms room air will enter the system through

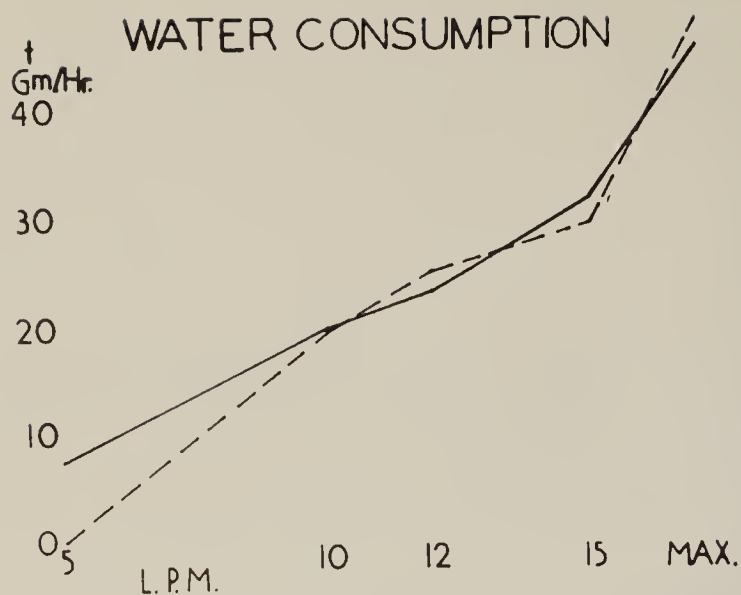


Fig. 1.—Aerosol output measured by weight of water consumed (solid line) and by densitometry (dashed line). At 5 L. per minute appreciable consumption by evaporation and small aerosol output account for the deviation at this flow. Densitometry output is calculated from the common logarithm of the ratio of light transmitted without and with the aerosol times a constant of 242.

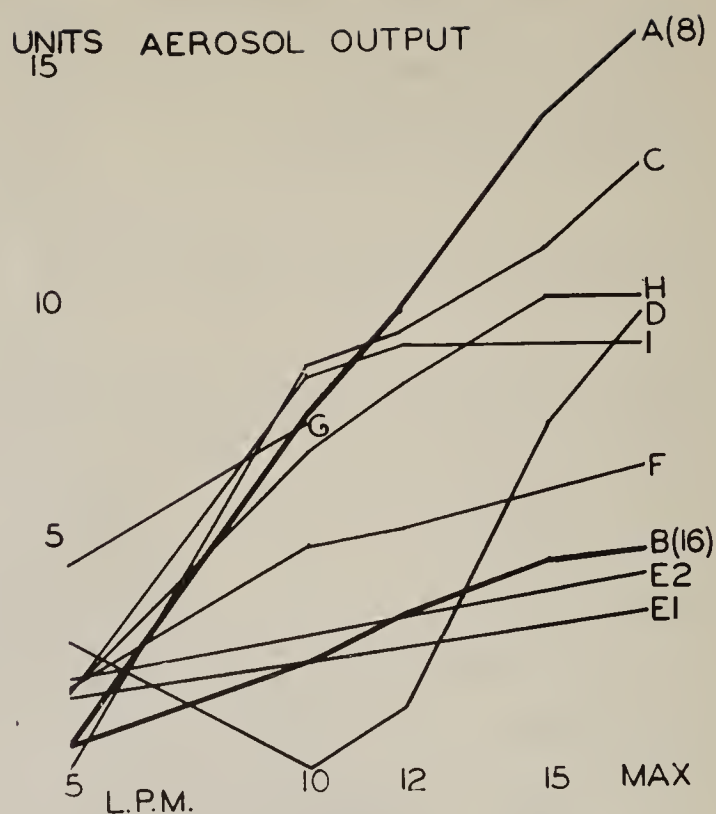


Fig. 2.—Aerosol outputs by densitometry of generators of various manufacturers and at various propelling oxygen flow rates without dilution. The heavy lines represent the average outputs of the number of generators in parenthesis. Units are arbitrary.



Fig. 3.—Aerosol outputs of the type A generators showing the uniformity of behavior.

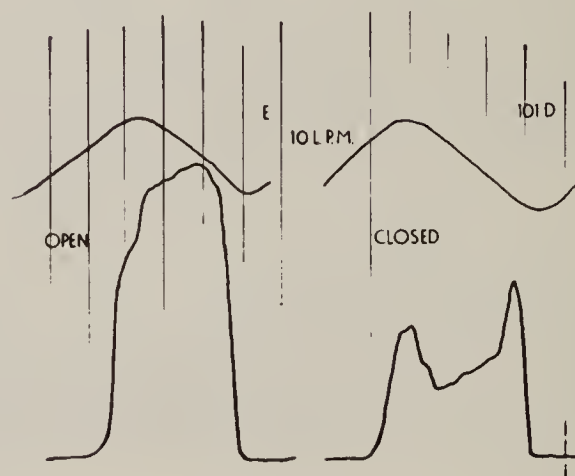


Fig. 4.—The quantity of aerosol inhaled as measured by densitometry with the diluter open and closed (lower record). Upper record is spirometric air flow produced by a mechanical respirator. Quantity of aerosol is indicated by the area under the curve. Recording is from right to left and vertical lines are at one second intervals.

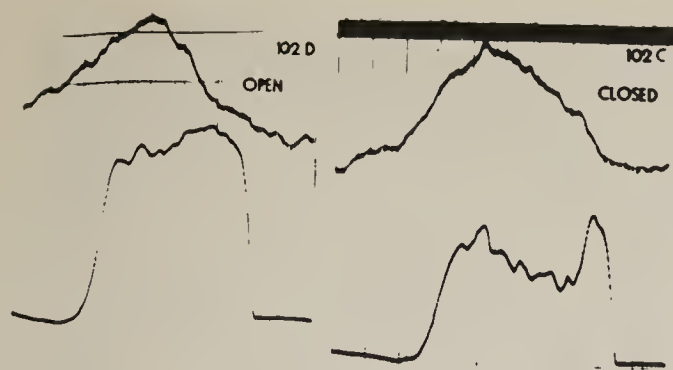


Fig. 5.—Record similar to Fig. 4 but using a trained subject. The areas under the lower curves show the marked increase in aerosol inhaled when the diluter is open.

holes in the face mask whenever peak inspiratory demand exceeds the rate of gas flow. Some of the aerosol will evaporate into this relatively dry room air, thereby reducing the aerosol content.

The variation of aerosol production by air dilution is related both to respiratory rate, and to tidal volume, as shown in Fig. 6.

One may achieve nearly the same increase in aerosol production without air dilution by using an air-tight mask of the reservoir type, with an inlet designed to fit the large-bore tube from the generator. During expiration, gas and aerosol collect in the reservoir bag, and are inhaled during the following inspiration, avoiding the use of outside air.

Increased aerosol dosage can be achieved by the addition of heat. The capacity of gas to hold water will increase as it is heated. When the gas and aerosol are delivered to the patient at room temperature water will evaporate from the aerosol as it is warmed to body temperature. To prevent this loss of water, the aerosol generator is heated to levels above body temperature. When the inhaled gas, so heated, is cooled to body temperature, precipitation of water occurs.

As each of these factors was recognized clinical results improved. Further effect was obtained by connecting two generators to one mask. With ultrasonic technique greater aerosol production occurs, and further improvement in aerosol therapy can be expected.

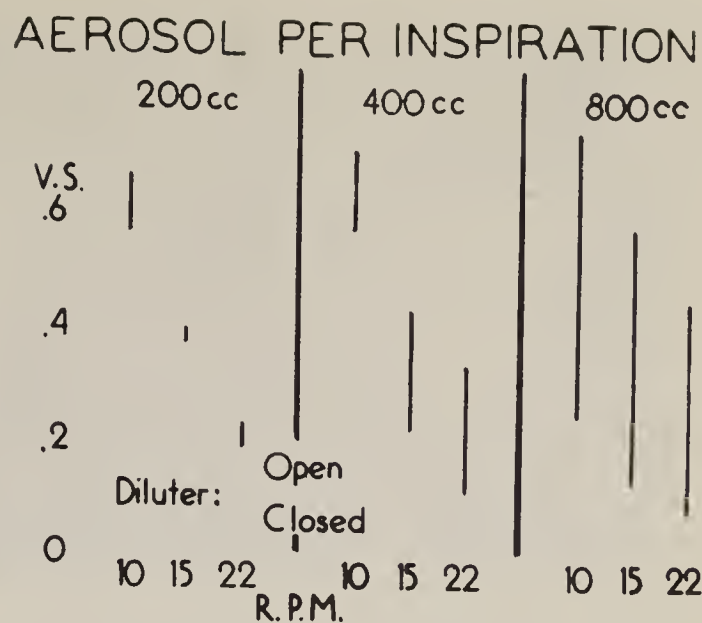


Fig. 6.—Aerosol "inhaled" at various rates and tidal volumes with the diluter closed (lower ends of vertical bars), and diluter open (upper ends). A mechanical respirator and spirometer are used to control rates and volumes.

DESCRIPTION

The ultrasonic aerosol generator consists of a power unit and an aerosol unit. The latter contains a nebulizing chamber, transducer, fluid reservoir, and air blower.

The power unit supplies an alternating current at 1,350,000 cycles per second. The ceramic transducer vibrates when subjected to electrical waves at this frequency. These vibrations are focused on the surface of the fluid in the aerosolizing chamber. The intense mechanical energy breaks up the water into tiny particles. The vibrations are transmitted to the chamber by water. This makes it possible to isolate the aerosolizing chamber from the transducer so the chamber may be sterilized by autoclaving or other methods and is not contaminated by the apparatus. Heat is produced, causing a temperature rise of 3 to 10° C.

The air blower carries the aerosol through the $\frac{3}{4}$ inch tube to the mask at a rate of 25 L. per minute which is usually sufficient to prevent air being drawn in through the openings in the mask. Another source of gas flow may be used. Audible noise is very low and rarely noticed.



Fig. 7.—Darkfield illuminated photographs of aerosol outputs of a type B generator (left) and of a type A (middle) of Fig. 2, and of the ultrasonic generator (right). Gas flows through the generators are identical and air dilution is absent. Photographic technique is the same for the three generators.

Four output levels are supplied of approximately 1, 2, 4, and 6 ml. per minute.

Particle size is ideal as indicated by absence of precipitation on the hand or on glass or metal surfaces at room temperature; and second, by exhalation of a negligible amount of the aerosol. Drop-out rate in a vertical tube, as measured by the optical densitometer, reveals good uniformity of particle size.

The maximum output of aerosol is over 6 ml. per minute. This is seven to ten times, or more, than the output of conventional aerosol generators (Fig. 7.). Heat is not used because the additional humidification is so slight compared to the aerosol output as to be negligible (25 L. dry air at 37° C. requires 1.1 ml. of water for saturation).

Electronic apparatus utilizing high frequency electromagnetic waves are controlled by the Federal Communications Commission to prevent interference with radar, radio and television. The ultrasonic generator has FCC approval, but the required filters produce a potential of 60 volts at a current of 0.2 milliamperes between the instrument case and ground, with the switch turned on or off and the case ungrounded. This is prevented by the use of three conductor cable for 110 volt 60 cycle current and ground.

TECHNIQUE

At the highest output level, water is irritating and will cause severe coughing; half-normal saline causes less coughing; normal saline is non-irritating and is used exclusively. Only experience at a high output level is reported in this study.

The aerosolizing chamber, solution reservoir, connecting tubes and mask are sterilized to prevent transport of viable microorganisms by the aerosol to the patient. Culture counts have not revealed any appreciable decrease in the number of bacteria in the aerosolizing chamber.

The cable is inserted into a three conductor 110 volt 60 cycle outlet. If an adaptor is used for a two conductor outlet, the ground wire is attached to the screw on the outlet box cover.

The usual duration of treatment is one half-hour, either as therapy or to obtain bronchial secretions for cytologic or bacteriologic study. If wheezing occurs, treatment is stopped. Slight increase in shortness of breath does not necessitate cessation, but patients are warned to stop if respiratory distress occurs. (See discussion.) Treatments are given from one to four times a day. Patients who tolerate therapy for only a few minutes are given

five-minute treatments as frequently as every hour.

Water will precipitate in the tubing and collect at the lowest point. If obstruction occurs, output is reduced and overheating follows. In the course of a thirty-minute treatment, the precipitated fluid may have to be drained two or three times. While this is disadvantageous, it is considered a natural phenomenon with the high aerosol output. The generator is placed below the mask level for recumbent, unconscious and tracheostomized patients, or when unattended.

RESULTS

Secretions for Cytological Examination: Seven patients had findings suggestive of bronchogenic carcinoma and had received conventional aerosol therapy with heated hypertonic solutions for the purpose of obtaining sputum specimens. With ultrasonic saline aerosol therapy, all patients produced more bronchial secretions and with less effort than with the conventional therapy. Two patients receiving ultrasonic saline aerosol therapy did not cough spontaneously, but did bring up about 5 ml. of secretions when directed to cough. The secretions in all patients were considered to be of bronchial origin because of the presence of pulmonary macrophages. With one exception, the patients preferred the ultrasonic saline aerosol therapy to the conventional type because it was more effective, less irritating, and did not cause as much coughing.

Postoperative Bronchostasis: E.B., a 51 year old female, had a thoracotomy and sleeve resection of the upper lobe of the right lung for bronchogenic carcinoma. Bronchoscopy was necessary on the second and third postoperative days because of bronchostasis. Ultrasonic saline aerosol therapy for five minutes every two hours on the fourth and fifth days caused productive coughing. Uneventful recovery followed.

Bronchial Asthma: R.T., a 64 year old female with bronchial asthma developed cardiac failure after an acute respiratory infection, and received digitalis, amino-

phylline, corticosteroids, and intermittent positive pressure breathing with isoproterenol for one week, without improvement. Increased sputum production and marked clinical improvement followed ultrasonic saline aerosol therapy.

M.G., a 45 year old female, had been observed at intervals since 1941 because of severe bronchial asthma, bronchiectasis, suppurative bronchitis, arthritis, pulmonary fibrosis and rheumatic heart disease. While hospitalized she developed a respiratory infection with severe dyspnea. Response to intermittent positive pressure breathing with acetyl cystine was unsatisfactory. Ultrasonic saline aerosol therapy, given for five minutes every two hours, was followed by increased sputum production, decreased effort of coughing, and reduction of dyspnea. Therapy was stopped after four days.

M.W., a 35 year old female, had bronchial asthma and bronchiectasis associated with bouts of fever occurring every three to five days. One-half hour of ultrasonic saline aerosol therapy caused increased sputum production, fever disappeared, and dyspnea improved. Complete relief occurred when food sensitivity was identified, and dietary control instituted.

Pulmonary Fibrosis: P.M., a 62 year old male, with pulmonary fibrosis, suppurative bronchitis, bronchiectasis, cor pulmonale, secondary polycythemia, was unable to obtain relief from cough and dyspnea with control of cardiac failure, and infection. Intermittent positive pressure therapy with humidification gave some relief. Ultrasonic saline aerosol therapy resulted in increased sputum production, and relief of dyspnea greater than obtained with intermittent positive pressure breathing or conventional aerosol therapy.

Diffuse Obstructive Pulmonary Emphysema: A.R., a 70 year old male, with obstructive emphysema was treated with intermittent positive pressure breathing with isoproterenol and ultrasonic saline aerosol therapy, alternating twice

weekly. During the first three weeks he noted greater and more prolonged effect from ultrasonic saline aerosol. The fourth treatment had little effect. Thereafter, intermittent positive pressure breathing was more effective.

J.G., a 69 year old male with emphysema, was able to tolerate conventional heated saline aerosol, but with little or no effect on dyspnea. Ultrasonic saline aerosol therapy was given for seven minutes, interrupted four times because of coughing. Increased quantities of sputum were produced, and dyspnea was reduced. Fatigue and respiratory distress due to coughing made it necessary to stop treatments.

F.R., a 59 year old male, treated with intermittent positive pressure breathing and isoproterenol at monthly intervals, supplemented by home therapy using isoproterenol by nebulizer and compressed air pump, found 30 minutes of ultrasonic saline aerosol therapy resulted in production of twice as much sputum, with relief of dyspnea exceeding that with isoproterenol aerosol or intermittent positive pressure breathing. Six months later he was hospitalized with cor pulmonale and cardiac failure. After one week, ultrasonic saline aerosol therapy was instituted, with increased quantities of sputum, and decreased dyspnea. Improvement was accelerated when ultrasonic saline aerosol therapy was started.

C.M., a 64 year old male with cor pulmonale, obstructive emphysema and severe dyspnea, found dyspnea increased to an intolerable extent and stopped ultrasonic saline aerosol therapy after 15 minutes. No improvement was observed.

DISCUSSION

Alveolar air at body temperature and

saturated with water is a prime requirement for normal diffusion of oxygen and carbon dioxide. Except during mouth breathing, the nose accomplishes the major warming and humidification. Some evaporation of water occurs in the tracheobronchial tree, particles of fluid migrating from the mucosa and its glands into the mucous sheath to replace water lost through evaporation. Evaporation increases the viscosity of the mucus; humidity therapy reduces evaporation thereby decreasing the viscosity. Aerosol therapy has the same effect, but, in addition, causes deposit of fluid particles on the mucous sheath, further decreasing viscosity and aiding bronchial drainage.

If the airway lumen is reduced to a critical point, water retained in the sheath and particles deposited on the sheath may decrease the lumen and cause increased respiratory obstruction. Patients are encouraged to continue treatment, and relief will occur when the secretions are coughed out or aspirated. Many patients tolerate aerosol therapy after bronchoscopy with irrigation and aspiration of secretion, but not before. If bronchostasis is the principal cause of obstruction, persistence in therapy is indicated.

Patients who have experienced intolerable dyspnea with ultrasonic aerosol therapy, but not with conventional aerosol therapy, have been included in this study as evidence of the greater deposit of fluid in critical areas of the air passages.

Our experience with detergent and enzyme aerosols indicates that large doses cause irritation. Low doses are better tolerated but are less effective than high doses of water or saline aerosol. For this reason such drugs and antibiotics have not been used in the ultrasonic generator.

SUMMARY AND CONCLUSIONS

1. Evaluation of aerosol therapy requires recognition of quantity (i.e. the dosage) of aerosol and is related to the type of generator, the flow of propelling

gas, the use of air dilution or reservoir type mask, and heat. Attention to these factors makes it possible to increase dosage and effectiveness of therapy.

2. An experimental aerosol generator, utilizing ultrasonic vibration, supplies over 6 ml. per minute of aerosol, compared to outputs of less than 1 ml. per minute with currently available generators. Electrical grounding and drainage of precipitated aerosol from the tubing to the mask are required. Very little audible noise is produced.

3. Preliminary study of the ultrasonic aerosol generator in the treatment of patients with suspected bronchogenic carcinoma, bronchial asthma, diffuse obstructive pulmonary emphysema, pulmonary fibrosis, and bronchostasis indicates the following:

- a. Administration of water by the ultrasonic generator causes severe coughing and irritation of the trachea and bronchi; normal saline causes less coughing and minimal irritation.
- b. For cytological studies ultrasonic saline aerosol therapy causes less coughing and less irritation, with ex-

pectoration of a larger quantity of bronchial secretion than conventional heated hypertonic saline aerosol therapy.

- c. Patients who experienced increased dyspnea with conventional aerosol therapy notice dyspnea sooner and more severely with ultrasonic saline aerosol therapy.
- d. Improvement in bronchial drainage is observed consistently in those patients with significant bronchostasis, providing that increased airway flow resistance of intolerable degree does not occur.

4. This preliminary study indicates that ultrasonic saline aerosol therapy is more effective than conventional aerosol therapy for reducing the viscosity of bronchial secretions, is a valuable therapeutic procedure, represents a new dimension in aerosol therapy, and warrants further study.

Acknowledgement

Experimental models of the ultrasonic generators were produced by the DeVilbiss Company, Toledo, Ohio.



NEOPLASMS OF THE LIVER AND URINARY BLADDER IN RATS FED 2-ACETYLAMINOFLUORENE AND INDOLE:

The effect of previous injections of Carbon Tetrachloride On The Neoplasms.

RYOICHI OYASU, M.D.¹

JAMES H. McDONALD, M.D.²

GEORGE M. HASS, M.D.³

As an occupational disease cancer of the urinary bladder is closely related to the development of the aromatic amine and azo-dye industry and to the subsequent exposure to these chemicals of a large number of workers.¹⁻³

In 1953 Clayson attributed carcinogenic action of aromatic amines to their conversion metabolically to o-hydroxyamines,⁴ which are concentrated in the urine and exert most of their carcinogenic effect on the bladder, where stasis of urine is a significant factor. Recently Miller, Miller and Hartman proposed a new hypothesis based on a study of 2-acetylaminofluorene (2-AAF) carcinogenicity.⁵⁻⁶ Although many metabolites were isolated, N hydroxy-2-acetylaminofluorene was the major metabolite in the urine of rats fed or injected with 2-AAF for over six weeks. This compound, when administered, is more active than 2-AAF as a carcinogen.

Experimental studies on bladder neoplasia in laboratory animals were greatly stimulated by the original report on 2-AAF by Wilson, DeEds and Cox in 1941.⁷ A large number of subsequent studies have demonstrated the marked potency and wide range of action of this compound which had induced tumors in many tissues of diverse species and strains. Attempts to produce bladder

tumors in various strains of rats with 2-AAF have met with some success.⁷⁻⁹ Dunning and Curtis showed that the incidence of bladder malignancies in 2-AAF fed Fischer line 344 rats could be strikingly increased by supplementing the diet with an excess of DL-tryptophan¹⁰ or indole.¹¹

Our interest in bladder neoplasia is ultimately oriented towards the problem of "spontaneous" bladder cancer in man. Although 2-AAF is unlikely to be encountered by most people, yet there is considerable justification for studying the action of 2-AAF and indole in rats. The structure and reactions of 2-AAF may have certain similarities to unknown carcinogens postulated to be responsible for most "spontaneous" bladder tumors in man. In addition, indole is a metabo-

From the Division of Pathology and the Division of Surgery

¹ Present address: Kyoto National Hospital
Fukakusa, Kyoto, Japan

² Attending Urologist, Presbyterian-St. Luke's Hospital; Professor of Urology, University of Illinois College of Medicine

³ Attending Pathologist, chairman of the division, Presbyterian-St. Luke's Hospital; Professor of Pathology, University of Illinois College of Medicine

lite of tryptophan, both being normal factors in human metabolism. If either can be demonstrated to have a definite role in bladder carcinogenesis in animals, a similar role might be active in man. Our experiments thus far have been of two types. In the first experiment rats were fed diets containing 2-AAF with or without supplemental indole. In the second experiment the 2-AAF feeding with or without indole was preceded by carbon tetrachloride injections in order to observe the effect of liver injury on the 2-AAF-indole tumorigenesis. In all experiments Charles River W (Wistar) male rats weighing 120 to 139 grams, approximately six weeks old, were used.

EXPERIMENT I

Animals were divided into four synthetic dietary groups, with diets generally patterned after those used by Dunning and Curtis^{10,11}: Group B (0.06% 2-AAF + 1.6% indole), Group D (3.2% indole), Group E (0.06% 2-AAF + 3.2% indole) and Group F (0.06% 2-AAF). Rats were killed at

intervals of three months. These together with those dying spontaneously were necropsied with microscopic examination of all organs. All animals dying before the sixth month were excluded from the results.

The potency of 2-AAF as a liver carcinogen, as well as a liver poison, was manifested to the greatest extent in Group F, in which there was an average survival time of only 194 days with no rats living longer than 234 days. (Table 1). When the diet was supplemented with excess indole, the average survival time was considerably prolonged. The survival time of other groups ranged from 337 to 430 days. Over 90% of rats of Groups B, E and F developed liver tumors with malignant tumors in Group F (100%), Group B (52%) and Group E (15%). The malignant hepatic tumors consisted predominantly of hepatic cell carcinomas with occasional cases of bile-duct cell carcinomas. The earliest and most extensive histological changes occurred in rats of Group F, and began during the third week of dietary treatment. These included the proliferation

TABLE 1
COMPARISON OF YIELD OF TUMORS IN LIVER AND BLADDER WITH
RESPECT TO CCl₄ PRETREATMENT

Group	No Administration of CCl ₄				Previous Administration of CCl ₄			
	2-AAF No indole (F)*	2-AAF 1.6% indole (B)*	2-AAF 3.2% indole (E)*	No 2-AAF 3.2% indole (D)*	2-AAF No indole (FT)*	2-AAF 1.6% indole (DT)*	No 2-AAF 3.2% indole (DT)*	No 2-AAF No indole (NT)*
No. of rats	26	31	27	33	13	34	5	22
Average survival, days	194	337	372	430	40 + (105-140 on CCl ₄)	327 + (105-140 on CCl ₄)	326 + (105-140 on CCl ₄)	325 + (105-140 on CCl ₄)
Liver tumors %								
Benign	0	48	78	0	0	41	0	0
Malignant	100	52	15	0	0	9	0	0
Bladder tumors %	19	58	44	0	0	6	0	0

*All rats dying within 5 months were excluded from table and results except Group FT where all rats died within 2½ months.

of ductal-type cells in the periphery of the lobule and the formation of multiple regenerated nodules of hepatic cells. The continued proliferation of the ductal-type cells developed into a glandular pattern and finally benign cystadenomas. The continued proliferation of regenerating hepatic nodules resulted in hepatic, and rarely duct-cell, carcinomas in the sixth month. Rapid development and high incidence of malignant tumors were characteristic of Group F (2-AAF), and a high incidence but delayed development of cystadenoma were characteristic of Group E (2-AAF + 3.2% indole).

In Group F, 5 out of 26 rats developed bladder tumors before the ninth month. In Groups B (2-AAF + 1.6% indole) and E (2-AAF + 3.2% indole) the development of bladder tumors was delayed up to the tenth month, but the incidence rose to 68% and 63% respectively between the 10th and 17th months. Histologically, multiple areas of focal hyperplasia of epithelium, transitional or squamous, appeared by the sixth month in Group F. This state rapidly progressed to a marked squamous metaplasia or a transitional cell papilloma. The development of similar changes was delayed in Groups B and E until the ninth month. After the tenth month, malignant bladder tumors had developed in 48% of Group B and 47% of Group E rats.

In Groups B, E and F, leukemias and varied malignant tumors in other organs were observed in low incidence. There were two cases of carcinoma of the renal pelvis. No neoplastic changes were observed in Group D.

Indole definitely prolonged the life of the animals, modified the damage to the liver, and increased the incidence of bladder tumors. The development of bladder tumors was frequently associated with extensive involvement of the liver by neoplastic processes. Using 50% as an arbitrary but convenient index value for classifying animals, the degree of liver damage in each rat of Groups B and E was estimated by gross and microscopic examination. As shown in Table 2, the

bladder tumor incidence was much higher in rats with less than 50% functional liver tissue. Within the limits of this type of study there appeared to be a strong positive correlation between extensive liver changes and bladder neoplasia.

EXPERIMENT 2.

The first experiment on the effects of excess dietary indole on 2-AAF neoplasia of the liver and urinary bladder indicated that the development of bladder tumors was positively correlated with severe non-malignant liver damage. If this relationship were a causal one, the development of bladder tumors might therefore be enhanced by damaging the liver by other means prior to the administration of 2-AAF. For this purpose, carbon tetrachloride (CCl_4) was used.

The animals were fed a stock synthetic diet, and each rat received a subcutaneous injection of 1 ml. of a solution containing 0.1 ml. of CCl_4 dissolved in 0.9 ml. of olive oil three times weekly. The first group of 55 rats received a total dosage per rat of 4.5 ml. of CCl_4 during a period of 15 weeks and the second group of 35 rats, a total of 6.0 ml. during a period of 20 weeks. At the end of these treatments, 7 rats from the first group were sacrificed and 5 showed mild cirrhosis. All 5 rats from the second group exhibited mild to moderate cirrhosis at necropsy examination. Centrilobular hydropic degeneration was present in all 12 rats. The remaining rats from both groups were arranged into 4 dietary groups; Group FT (2-AAF), Group BT (2-AAF + 1.6% indole), Group DT (3.2% indole) and Group NT (stock synthetic diet). In all groups the animals gained weight progressively and had a higher average weight than animals in corresponding groups (Groups F, B, and D) of the previous study. At the end of 12 months on dietary treatment all rats were killed. These, together with those dying spontaneously were necropsied and sections were taken as in the previous study. Except for Group FT (2-AAF), all rats dying within the first 5

TABLE II
LIVER DAMAGE VS. BLADDER TUMOR IN RATS SURVIVING OVER NINE MONTHS

ESTIMATED AMOUNT OF FUNCTIONAL LIVER TISSUE	Group					
	B		E		Total (B + E)	
	No. of Rats	Bladder Tumor	No. of Rats	Bladder Tumor	No. of Rats	Bladder Tumor
More than 50%	6	2 (33%)	7	0	13	2 (15%)
Less than 50%	19	12 (63%)	15	11 (73%)	34	23 (68%)

months of the dietary treatment were excluded from the results shown in the table.

All rats of Group FT died of severe liver damage within 66 days on their diet (0.06% 2-AAF), the average survival time being 40 days (Table 1). The other groups had longer average survival times that ranged from 325 to 327 days. Compared with the results of the previous experiment, there was a marked decrease in the incidence of neoplasm in all sites in the present study. Liver tumors occurred only in Group BT with an incidence of 50%. Hepatomas developed only in 9% and none of these were seen before the 10th month of the dietary treatment. Although no neoplastic changes were evident, the morphologic changes of the liver by dietary treatment were most severe in Group FT (2-AAF) and the cirrhosis induced by CCl₄ was exaggerated by the end of the first month of the subsequent 2-AAF administration. Small veins and capillaries connecting the portal veins to the central veins induced by CCl₄ administration were constantly seen. A uniform distribution of bile-duct type cells within the pseudolobules as well as in the pre-existing portal triads was a striking picture. Scattered through the fields were few nodules of apparently regenerated hepatic cells. These changes were minimal in those which received indole. CCl₄-induced cirrhosis persisted but was minimal with very slight fatty metamorphosis. Slight irregularity of hepatic cells and rare regenerated nodules were the only positive findings within the lobules. The proliferation of duct-cells was limited only to a few portal spaces.

In only 41% of animals, however, was the change sufficient to be classified as cystadenoma.

The urinary bladder was the site of tumor in only 2 rats, both of which were in Group BT. One was a papilloma occurring in the 9th month and the other was a papillary transitional cell carcinoma showing early invasion. Other tumors such as cerebral gliomas were also observed in a low incidence in Group BT. In Group NT there were four retroperitoneal sarcomas. These were presumed to be directly related to local injurious effects of CCl₄ injections. No neoplastic or proliferative changes in the liver were observed in Groups DT and NT.

Carbon tetrachloride pretreatment thus appears to have markedly reduced the efficacy of 2-AAF and indole for induction of tumors of the liver and urinary bladder. However, as shown by a comparison of Group F and Group FT, (both on 2-AAF alone) the rate and extent of toxic injury from 2-AAF were apparently increased by CCl₄ pretreatment as evidenced by the early appearance of marked histological changes in the liver and early death of all animals in Group FT. With excess indole in diet this exaggerated toxic effect was prevented or markedly lessened, and histological changes in the liver were much milder in Group BT than those found in Group B rats. Thus, a protective action of indole was strongly accentuated in the present study.

The means by which indole-tryptophan enhances bladder tumorigenicity is not known. Certain tryptophan metabolites are considered to be responsible,

and even have been considered to be possible carcinogens, for "spontaneous cancers" in man.¹²⁻¹⁶ This is highly unlikely in view of the fact that none of our rats taking the high indole diet (Group D) developed bladder tumors. Dyer and Morris found an apparent interference between pyridoxine and 2-AAF (or a metabolite) (quoted by Weisburger and Weisburger).¹⁷ A challenging dose of tryptophan in rats ingesting 2-AAF had a pronounced influence on tryptophan metabolism resulting in excretion of much more xanthurenic acid than by the controls not fed the carcinogen. Thus, as Weisburger and Weisburger suggested,¹⁷ there may occur in the liver competitive displacement of the fluorene metabolites by tryptophan or indole metabolites, manifesting itself by an increased latent period in the induction of liver tumors but a high incidence of liver and bladder tumors later on.

On the other hand, the increased survival time of indole-fed rats may have been the primary factor by which indole increased the incidence of bladder tumors in this study. The early appearance of pre-neoplastic lesions in the bladder (multiple areas of focal hyperplasia) indicates that the rats fed 2-AAF alone might have developed more bladder tumors if they had lived longer than seven months.

In our second experiment, however, in spite of approximately same average survival times of Groups B and BT, the incidence of tumors decreased markedly in the rats that received CCl₄ injections. The livers of these animals at the completion of CCl₄ injections revealed numerous microscopic Eck fistulae connecting the portal vein system to central veins, and therefore much of the portal blood by-passed without being exposed to the liver parenchyma. This could result in decreased production of carcinogenic metabolites. However, in Group FT, even this small amount of 2-AAF available to the liver cells seemed to be sufficient to produce severe toxic injury to a liver which had already been damaged by preceding CCl₄ injections.

In rats taking diet supplemented with indole this small amount of 2-AAF could easily be removed by competitive displacement by indole metabolites.

A similar inhibitory effect of cirrhosis was observed by Dyer.¹⁸ She administered butter yellow to rats and reported the slower development of liver tumors in rats on a low choline diet with cirrhosis than in those without cirrhosis on a choline-supplemented diet.

The correlation between the liver injury and bladder tumors in the first experiment was neither substantiated nor contradicted in the second experiment. Further studies are being conducted on this subject.

SUMMARY

Tumors often developed in the liver and less frequently in the urinary bladder of rats maintained for several months on a basal diet containing 2-acetylaminofluorene (2-AAF). If indole was added to this carcinogenic diet the tendency for tumors to appear in the liver was lessened, the animals lived longer and a greater incidence of bladder tumors was noted. Inasmuch as the earliest effects of the carcinogen were manifest by degeneration and repair of the liver, it was assumed that production of degeneration and repair of the liver by use of carbon tetrachloride prior to ingestion of the carcinogen would enhance the action of the carcinogen. This was the observed effect when the carcinogenic diet contained no indole and all animals died in a short time as a result of the toxic action of 2-AAF in the presence of an already damaged liver. However, when indole was added to the carcinogenic diet, the animals lived longer and displayed a high resistance to the action of the carcinogen in all locations. It seemed that this unpredicted and anomalous result might be attributed to the development of a carbon tetrachloride induced cirrhosis with its accompanying intrahepatic portocaval shunts. The shunts in turn could conceivably have created a by-pass so that less of the oral precursor carcinogen was directly converted into

actual carcinogens by hepatocellular metabolic actions. An alternative equally tenable speculation is that hepatocellular

systems responsible for metabolizing 2-AAF into carcinogenic compounds were inactivated by carbon tetrachloride.



REFERENCES

1. Case, R. A. M., Hosker, M. E., McDonald, D. B., and Pearson, J. T.: Tumors of the Urinary Bladder in Workmen Engaged in the Manufacture and Use of Certain Dyestuff Intermediates in the British Chemical Industry, Part I. The Role of Aniline, Benzidine, Alpha-naphthylamine, and Beta-naphthylamine, *Brit. J. Indust. Med.* 11:75-104, 1954.
2. Melick, W. F., Escue, H. M., Naryka, J. J., Mosera, R. A., and Wheeler, E. P.: The First Reported Cases of Human Bladder Tumors Due to a New Carcinogen—Xenylamine, *J. Urol.* 74:760-766, 1955.
3. Melick, W. F., and Naryka, J. J.: Xenylamine (para-aminobiphenyl) Bladder Tumors in Man, *Acta Unio Internat. contra Cancrum* 16:277-283, 1960.
4. Clayson, D. B.: A Working Hypothesis for the Mode of Carcinogenesis of Aromatic Amines, *Brit. J. Cancer* 7:460-471, 1953.
5. Miller, G. A., Cramer, J. W., and Miller, E. C.: The N- and Ring-hydroxylation of 2-Acetylaminofluorene during Carcinogenesis in the Rat, *Cancer Res.* 20:950-962, 1960.
6. Miller, E. C., Miller, G. A., and Hartmann, H. A.: N-Hydroxy-2-Acetylaminofluorene: A Metabolite of 2-Acetylaminofluorene with Increased Carcinogenic Activity in The Rat, *Cancer Res.* 21:815-824, 1961.
7. Wilson, R. H., DeEds, F., and Cox, A. J., Jr.: The Toxicity and Carcinogenic Activity of 2-Acetylaminofluorene, *Cancer Res.* 1:595-608, 1941.
8. Cox, A. J., Jr., Wilson, R. H., and DeEds, F.: The Carcinogenic Activity of 2-Acetylaminofluorene, Characteristics of the Lesions in Albino Rats, *Cancer Res.* 7:647-657, 1947.
9. Dunning, W. F., Curtis, M. R., and Madsen, M. E.: The Induction of Neoplasms in Five Strains of Rats with Acetylaminofluorene, *Cancer Res.* 7:134-140, 1947.
10. Dunning, W. F., and Curtis, M. R.: The Role of Dietary Tryptophane in the Incidence of 2-Acetylaminofluorene-induced Bladder Cancer in Rats, *Acta Unio internat. contra Cancrum* 11:654-657, 1955.
11. Dunning, W. F., and Curtis, M. R.: The Role of Indole in Incidence of 2-Acetylaminofluorene-induced Bladder Cancer in Rats, *Proc. Soc. Exper. Biol. & Med.* 99:91-95, 1958.
12. Allen, M. J., Boyland, E., Dukes, C. E., Horning, E. S., and Watson, J. G.: Cancer of the Urinary Bladder Induced in Mice with Metabolites of Aromatic Amines and Tryptophan, *Brit. J. Cancer* 11:212-228, 1957.
13. Boyland, E., and Watson, G.: 3-Hydroxyanthranilic Acid, A Carcinogen Produced by Endogenous Metabolism, *Nature* 177:837-838, 1956.
14. Boyland, E., and Williams, D. C.: 34th Rept. *Brit. Exp. Cancer Campaign*, 1956, p. 39.
15. Boyland, E., and Williams, D. C.: The Metabolism of Tryptophan, *Biochem. J.* 64:578, 1956.
16. Brown, R. R.: The Isolation and Determination of Urinary Hydroxynurenine, *J. Biol. Chem.* 227:649-652, 1957.
17. Weisburger, E. K. and Weisburger, J. H.: Chemistry, Carcinogenicity and Metabolism of 2-Fluorenamine and Related Compounds, *Advances in Cancer Res.* V, pp. 372-373, 1958.
18. Dyer, H. M.: The Effect of Supplementary Choline on the Livers of Rats Fed p-Dimethylaminoazobenzene, *J. Nat. Cancer Inst.* 11:1073-1080, 1951.

REVIEW ARTICLE

ANTICOAGULANT THERAPY: Theoretical and Practical Aspects

JOHN H. OLWIN, M.D.¹

JOHANNES L. KOPPEL, PH.D.²

INTRODUCTION

Anticoagulant therapy in its present form has been used for approximately two decades. As the term implies, it is the cautious interference with the normal or perhaps abnormal clotting mechanism in an attempt to lower the clotting tendency of the patient's blood without contributing to unusual bleeding. The ideal method of anticoagulant therapy has not as yet been reached, but there have developed during the last two decades routines which in the hands of experienced clinicians have given a certain amount of protection to individuals with various types of thromboembolic conditions. It is the purpose of this paper to present certain theoretical and practical aspects of anticoagulant therapy as they are understood at the present time.

TYPES AND PROPERTIES OF ANTICOAGULANTS

As at the beginning of the practical era of anticoagulant therapy, there are still only two main types of anticoagulants available to the clinician; 1) those with heparin-like properties, and 2) the so-called prothrombin depressants.

Of the first group heparin is the only drug of importance since the adminis-

tration of other materials such as Treburon, Paritol, Thrombocid and a number of other more recently developed drugs is accompanied by such distressing side reactions that they are not suitable for clinical use. Heparin is a refined tissue extract made, for the most part, from beef lung or intestine. In the body it occurs in the mast cells which line the capillaries of various organs and, although it is known to be a mucopolysaccharide, its precise chemical formula is still unknown. It has many actions, including that of being a strong anticoagulant, a good diuretic, an antiphlogistic, and an antihistamine. It is also an activator of lipoprotein lipase. It is inactivated by the gastric and intestinal secretions and, hence, must be given parenterally. As it is most readily available to the blood and its effects are more accurately determined by intravenous injection, this is the avenue of administration we prefer. Since it has a very marked effect on results of the pro-

From the Coagulation Research Laboratory,
Division of Surgery

¹ Attending Surgeon, Presbyterian-St. Luke's Hospital; Clinical Professor of Surgery, University of Illinois College of Medicine

² Associate Attending Biochemist in Surgery, Presbyterian-St. Luke's Hospital; Assistant Professor of Biological Chemistry in the Department of Surgery, University of Illinois College of Medicine

The studies on which this paper is based were supported in part by the Medical Research and Development Board, Office of the Surgeon General, Department of the Army, under Contract No. DA-49-193-MD-2171.

thrombin assays as they are now performed, when it is given in conjunction with prothrombin depressant drugs it should, with rare exceptions, be given only intravenously.

Prothrombin depressants are of two main types, those of coumarin origin—dicumarol, cyclocumarol, tromexan, liquamar, warfarin, and sintrom,—and those of indanedione origin—phenylindanedione, dipaxin, chlorphenylindanedione, bromindione and miradon. All of them can perhaps better be classified into three groups according to the rate at which they produce their action on coagulation factors. There are those with a slow induction and a slow recovery period, dicumarol and cyclocumarol. Secondly, there are those with a short induction period and a short recovery period, tromexan, phenylindanedione, sintrom and miradon. Thirdly, there are those with a short induction period and long recovery, dipaxin, warfarin, liquamar, chlorphenylindanedione and bromindione. The latter group may provide in many instances for a more stable and uniformly controlled “therapeutic

bracket”, while the second group provides for greater maneuverability of the therapy. Those in the first group, dicumarol and cyclocumarol, provide no advantage except that of known safety and dependability. Table I includes a list of the various drugs and certain characteristics of therapeutic significance.

INDICATIONS FOR ANTICOAGULANT THERAPY

It is not within the scope of this paper to deal exhaustively with the subject of indications. Since these may vary considerably with the thinking of different clinicians, and since space does not permit presentation of various views on the subject, we shall be satisfied at this point with the general statement that anticoagulant therapy should be used in most thromboembolic conditions where such therapy is not specifically contraindicated. Such conditions include thrombophlebitis, phlebothrombosis, pulmonary embolism, coronary thrombosis, cerebral thrombosis (where this can reasonably be distinguished from cerebral hemorrhage), central retinal vein

TABLE I

Drug	Induction Period (Days)	Recovery Period (Days)	Usual Loading Dose (mg.)	Usual second Dose (mg.)	Average Daily Dose (mg.)	Neutralizing Drugs; Vitamin K	
						Water-Soluble	Fat-Soluble
Dicumarol	3-5	4-10	300	200	25-75	+	+
Tromexan	2-3	2-4*	1800	1200	300-600	+	+
Cyclocumarol	2-5	4-14	200	100	12.5-50	+	+
Phenylindanedione	2-3	2-4*	300	200	50-100	+	+
Phenprocoumon**	2-3	7-14	21	15	1.5-4.5	+	+
Dipaxin	2-3	10-15	30	15	2.5-7.5	0	+
Warfarin	2-3	8-12	100	50	2.5-7.5	+	+
Acenocoumarin***	2-3	2-4*	28	16	2-8	+	+
Anisindione****	3-4	2-5*	400	300	50-150	0	+
Chlorphenindione	2-4	5-12	16	8	1-16	?	+
Bromindione	2-3	3-10	14	4	1-4	?	+

*Withdrawal of these drugs is often followed by a rise in the prothrombin levels to above 30 per cent of normal, the upper level of the therapeutic bracket, within 24 hours.

If fat-soluble Vitamin K (K₁) is given because of mild bleeding, it is wise to use small amounts (10 to 15 mg.) daily to prevent extreme “escape” from the therapeutic prothrombin bracket and resistance of the patient to further anticoagulant therapy.

**Phenprocoumon (Liquamar)

***Acenocoumarin (Sintrom)

****Anisindione (Miradon)

thrombosis, and peripheral arterial embolism. It may be administered prophylactically to patients with a history of thromboembolism who may be confined to bed for a protracted period of time, who may be undergoing surgery of various types, or who may have suffered injury of a severe degree. Prophylactic therapy is also indicated in patients with recurring episodes of thromboembolism of various types, such as thrombophlebitis migrans, and one or more episodes of peripheral arterial embolism.

THE BLOOD COAGULATION MECHANISM

A proper understanding of anticoagulant therapy is dependent on a knowledge of the mechanism by which blood clots. According to the Morawitz theory (1), blood clotting takes place in two main stages. In the first stage, prothrombin is converted to thrombin in the presence of thromboplastin, calcium, and the more recently discovered accelerators—factors V (Ac-Globulin, pro-accelerin, labile factor), VII (SPCA, pro-convertin, stable factor) and X (Stuart-Prower factor). In the second stage, thrombin converts fibrinogen to fibrin. The formation of intrinsic plasma thromboplastin may be looked upon as a third (or first) stage. Thromboplastin consists of tissue juice or, in blood free of tissue juice, results from the interaction of platelet phospholipid, Hageman factor (factor XII), plasma thromboplastin antecedent (PTA, factor XI), plasma thromboplastin component (PTC, factor IX), antihemophilic globulin (AHG, factor VIII) and calcium. Certain anticlotting factors are present in the blood, serving to maintain a balance between excessive clotting and excessive bleeding. Of these, fibrinolysin (plasmin) is important in the lysis of the clot, a reaction which may be looked upon as the fourth stage of the clotting process.

Heparin acts at a number of points in the clotting mechanism, particularly interfering with the conversion of prothrombin to thrombin, through its neutralization of intrinsic thromboplastin,

by inhibiting the action of thrombin on fibrinogen, and by aiding in the lysis of the newly formed thrombus.

The *in vivo* actions of both the coumarins and indanediones appear to be grossly similar in nature in that both types of drugs depress the syntheses of prothrombin, factor VII, factor IX, and factor X. However, there are differences between the various individual drugs in (1) the rates at which they reduce the plasma concentrations of these clotting factors, (2) the actual levels to which the factors are reduced, and (3) the rates at which the factors return to normal following the withdrawal of the drug.

METHODS OF CONTROL

As with any type of treatment, the value of anticoagulant therapy is materially dependent on an accurate method of its control both from a point of view of effectiveness and of safety.

Heparin therapy is readily controlled by means of the Lee-White (2) whole blood coagulation time whereas prothrombin depressant administration is almost universally controlled by one or more variations of the one-stage prothrombin ("prothrombin time") assay described originally by Quick (3). While this was considered originally by him to be a specific measure for plasma prothrombin, subsequent experience has shown that the prothrombin time is also a function of other variables. A further complicating feature of this test is the recent discovery that the various thromboplastin preparations used in the assay, all of which are of animal or human tissue origin, contain varying amounts of factor V and particularly factor VII (4). Thus, in the one-stage assay, one may be adding to the test system some of the very factors which he is assaying. These differences in the thromboplastins themselves are, we believe, at least in part responsible for the varying prothrombin results obtained from time to time (Fig. 1) and, hence, the resulting occurrence of unexpected hemorrhages in some cases and the apparent failure of anticoagulant therapy in others. Such

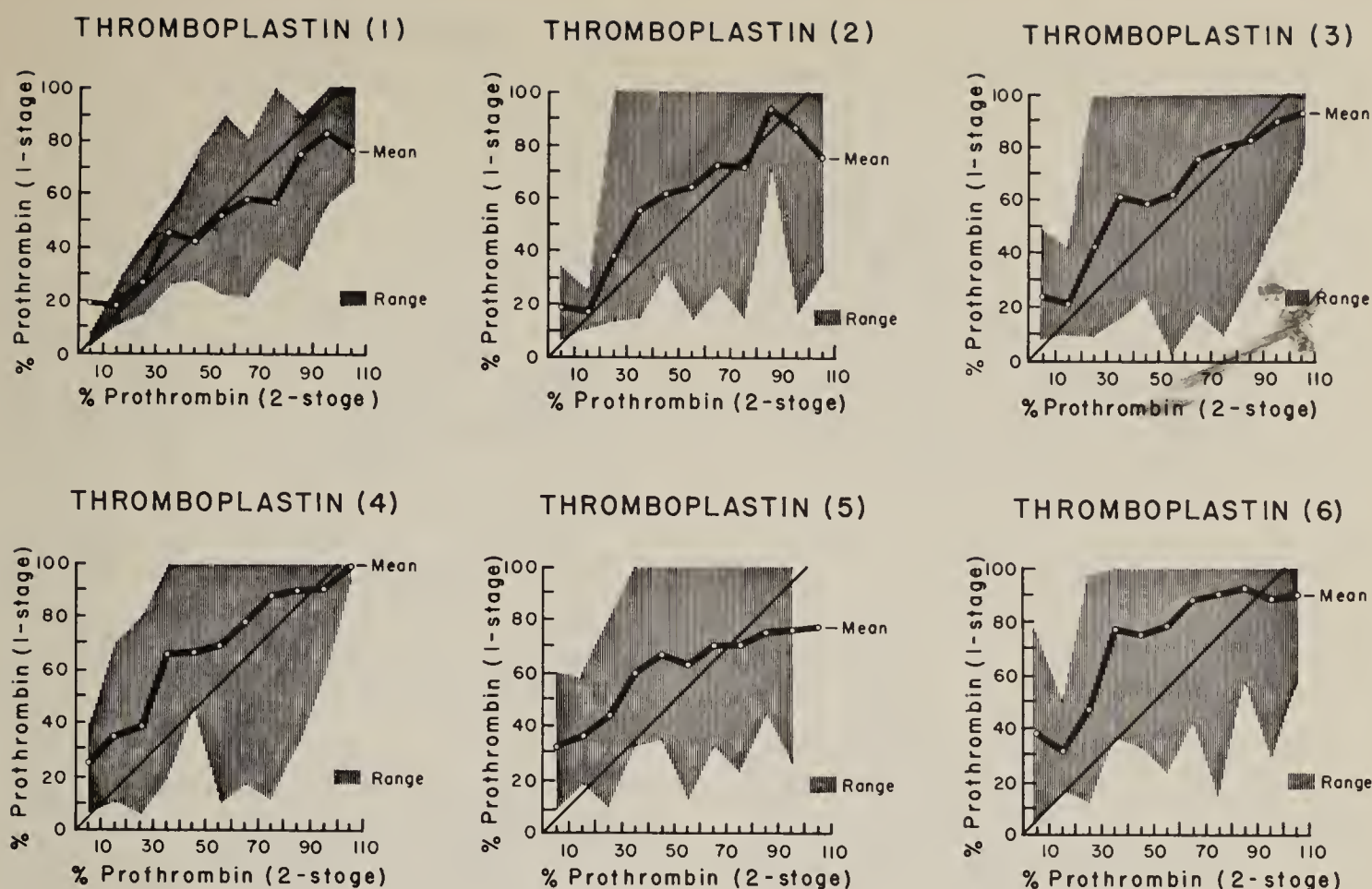


Fig. 1.—Prothrombin values with individual thromboplastins. The data presented for thromboplastins 1-6 were collected and processed in the following manner: (a) On the basis of its two-stage prothrombin level each plasma was assigned to a group arbitrarily designated as follows: 1-10 per cent, 11-20 per cent, 21-30 per cent, etc. (b) Using six different thromboplastins, one stage assays were run, in turn, on 12 to 15 plasmas comprising each group. (c) The average one-stage results for the plasmas within each group were plotted against the mid-point of its designated group: 5 per cent, 15 per cent, 25 per cent, etc. This is the mean curve. The spread (range) of the one-stage values was indicated at the same points. (d) The straight line represents the theoretical curve which would result if the one-stage and two-stage values were identical and is included for reference purposes.

Reprinted from "Differences in Tissue Thromboplastins and their Influence on the Control of Anticoagulant Therapy" by J. L. Koppel, P. M. Arscott, L. H. Friedman and J. H. Olwin, which appeared in *Surgery* 43: 94, 1958, through the courtesy of The C. V. Mosby Company.

often unpredictable clinical effects are probably largely responsible for the wide difference of opinion as to the safety and efficacy of these drugs. In this connection the availability of the two-stage prothrombin assay (5) and the TAME method (6) adds appreciably to the ease of control of this form of therapy. The results of these two procedures closely parallel each other (Fig. 2), and while they are also influenced by the additions of factors V and VII in the particular thromboplastin used, both methods separate the two stages of

the clotting mechanism, and practical experience has shown that they produce considerably less variable prothrombin results than the one-stage technique (6, 7, 8, 9) (Figures 3 and 4). We have used a combination of one-stage and two-stage tests for over a decade with gratifying results, and, more recently, have substituted a modification of the earlier TAME assay for the original two-stage procedure (10). Whatever method or methods of control are used, their constant close surveillance is essential to safe and efficient therapy.

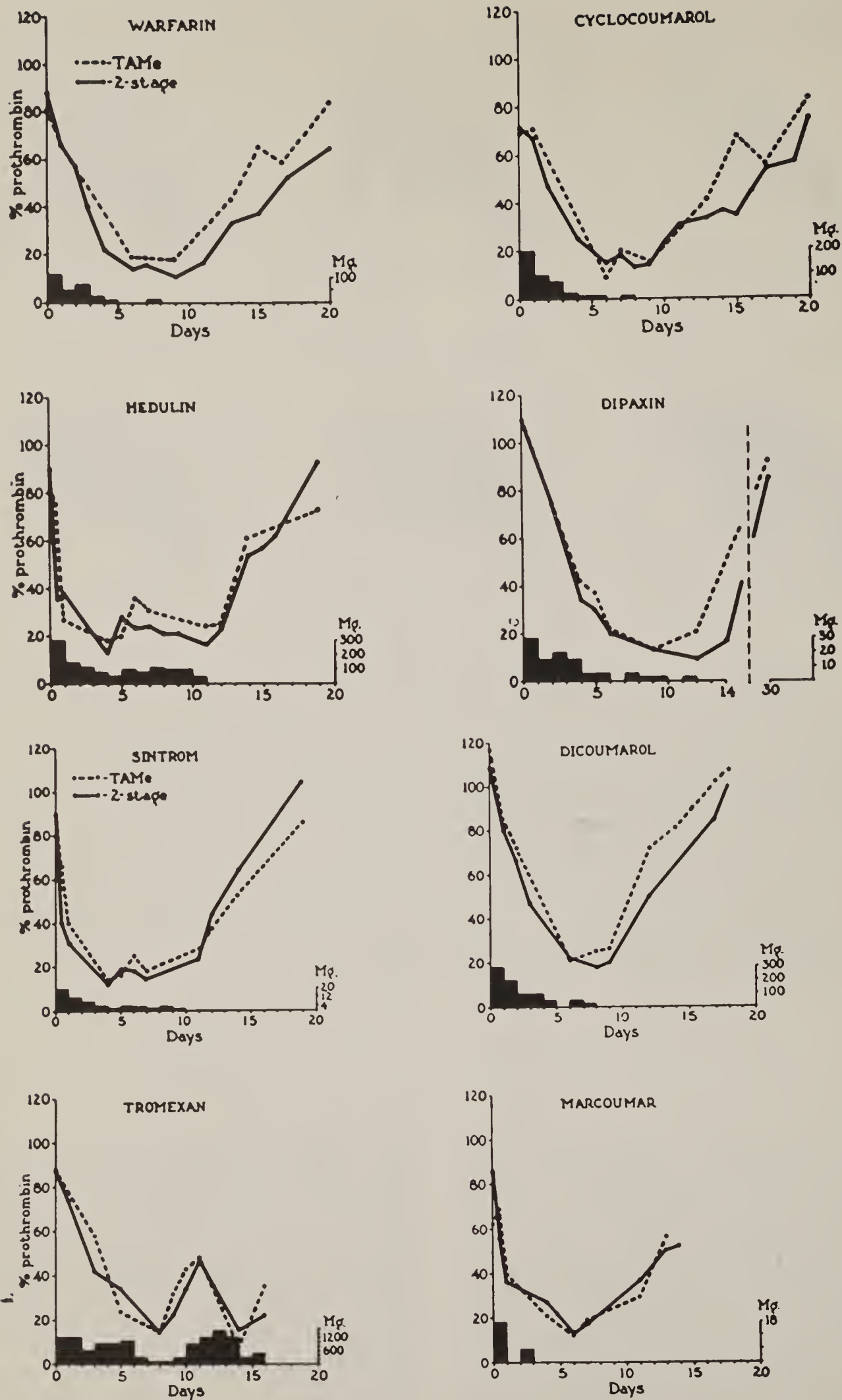


Fig. 2.—Two-stage and TAME prothrombin curves: Patients on eight different prothrombin depressant drugs.

Reprinted from "Experience with the TAME Assay During Anticoagulant Therapy" by Phyllis M. Arscott, J. L. Koppel, and John H. Olwin, which appeared in *The Journal of Laboratory and Clinical Medicine* 51: 805, 1958, through the courtesy of The C. V. Mosby Company.

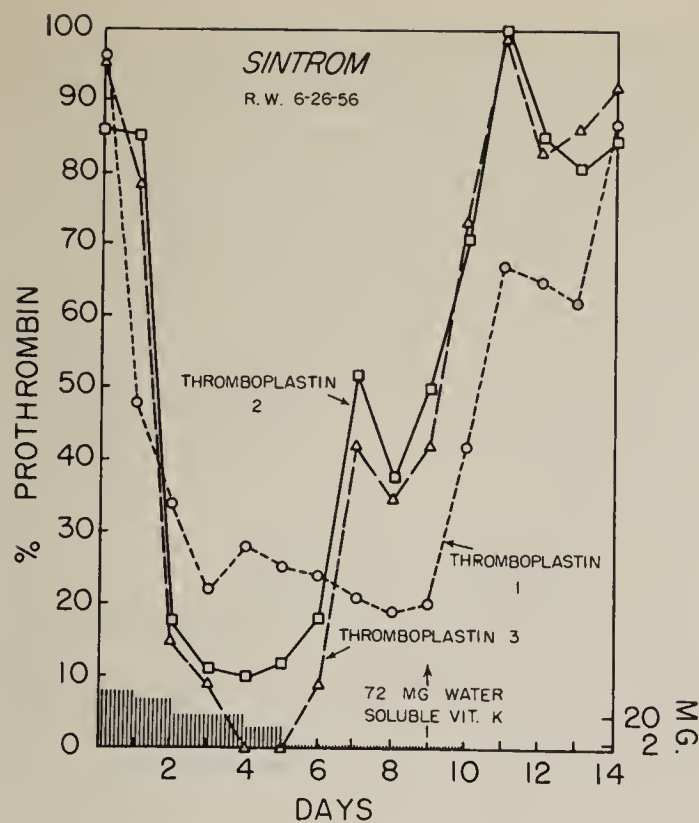


Fig. 3.—One-stage prothrombin curves resulting from Sintrom administration to a single patient. The three curves show the variation in the one-stage assay results when three different commercial thromboplastin preparations were used in the assay.

Reprinted from "Differences in Tissue Thromboplastins and their Influence on the Control of Anticoagulant Therapy" by J. L. Koppel, P. M. Arscott, L. H. Friedman and J. H. Olwin, which appeared in *Surgery* 43: 94, 9158, through the courtesy of The C. V. Mosby Company.

The commonly used one-stage test involves the addition of tissue juice (thromboplastin) to oxalated or citrated plasma, subsequent recalcification, and measuring the time required for the appearance of fibrin. Thus, the two stages described by Morawitz proceed simultaneously in the same test tube. It has been shown that a normal "prothrombin time" may be obtained when as little as 30 per cent of the available fibrinogen has been converted to fibrin. Some laboratories report their results in seconds while others prefer to convert the observed time to "percentage of prothrombin." In our opinion, the latter manner of reporting is preferable, since (a) it places the burden of accuracy on the laboratory, (b) it provides for less variation in interpretation, and (c) it provides for easier control when the patient moves from one geographical area to another and blood samples are processed by different laboratories.

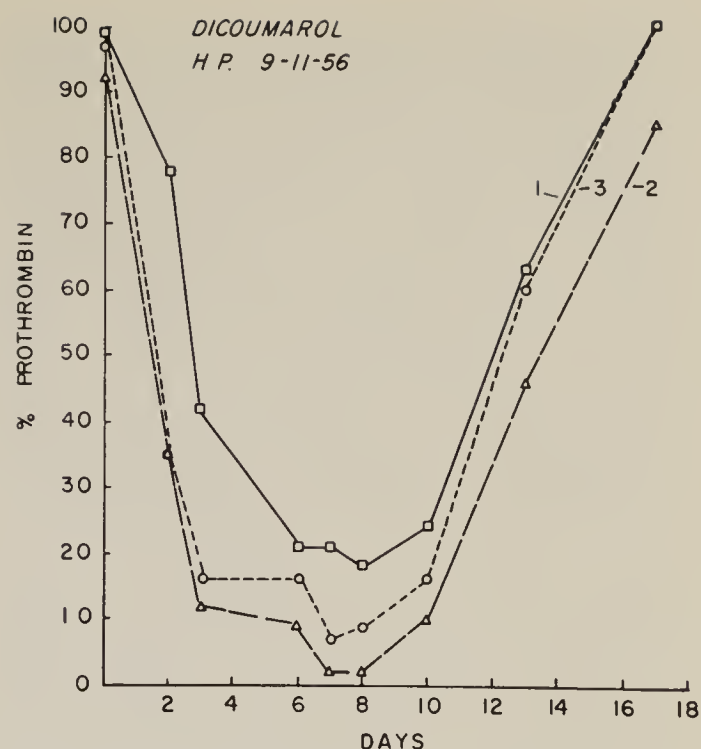


Fig. 4.—TAME prothrombin curves resulting from Dicoumarol administration to a single patient. The three curves show the variation in the TAME assay results when three different commercial thromboplastin preparations were used in the test. Reprinted from "Differences in Tissue Thromboplastins and their Influence on the Control of Anticoagulant Therapy" by J. L. Koppel, P. M. Arscott, L. H. Friedman and J. H. Olwin, which appeared in *Surgery* 43:94, 1958, through the courtesy of The C. V. Mosby Company.

ROUTINE OF ADMINISTRATION

When a decision has been made to place a patient on anticoagulant therapy and it has been established that there are no or only relative contra-indications to such, blood is drawn for coagulation time and prothrombin determinations. It is our policy to use two syringes for the drawing of blood since any tissue juice which may get into the blood sample may vitiate the results of both assays. One ml. of blood is drawn into the first syringe, it is detached and discarded and the blood is collected into the second syringe. When the blood has been drawn, heparin may be given through the same needle. The usual starting dose is 5,000 units (50 mg.). Since heparin given intravenously becomes effective almost immediately, sometime between 15 and 30 minutes after its injection another coagulation time is determined. It is our habit to titrate the coagulation time to between

two and three times the control reading by adjusting the heparin dosage. Since heparin is a relatively safe drug, greater increases within reason are probably without danger. The half life of heparin, given intravenously, is, in most individuals, approximately one hour. The effects of a single dose can usually be demonstrated in most patients to some extent up to six hours, but the coagulation time has usually returned to the control level by four hours. It is, therefore, our habit to repeat the heparin administration at intervals of four hours, and the proper dose may be adjusted by checking the coagulation time prior to and just following the heparin administration at these four hour intervals.

As soon as the results of the prothrombin determination are known, a loading dose of one of the prothrombin depressant drugs is given. Since little effect on the prothrombin level is shown in the first 36-48 hours of therapy, it is our practice not to repeat the prothrombin determination until the second day after the prothrombin depressant is started. This provides for an uninterrupted heparin routine for this period of time. On the second day following the institution of prothrombin depressant therapy, the usual heparin dosage in the morning is withheld for two hours, which provides a six hour period between the last dose of heparin and the drawing of blood for prothrombin determination. After the blood has been drawn, heparin administration is resumed and continued again at four hour intervals. This provides a brief period in which the patient may be only partially covered therapeutically but, empirically, this routine has proved satisfactory and without undue hazard. This procedure is repeated daily and heparin is continued until the patient's plasma prothrombin has been depressed to the control bracket (10 to 30 per cent of normal). This usually requires 3 to 5 days. Recent developments suggest that it may be plasma thromboplastin component (PTC) which is important in the thrombotic tendency and that it usually requires a minimum of five days for this

factor to be affected by prothrombin depressant drugs. It has been observed that the prothrombin, as measured by the one-stage assay, drops more rapidly than would be indicated by the two-stage test. This difference is thought to be due to the fact that factor VII which is depressed more rapidly than prothrombin is uncontrolled in the one-stage assay. If the dose of the prothrombin depressant is selected on the basis of the one-stage assay result, the attainment of the therapeutic bracket may be delayed by several days. If, on the other hand, the results of the two-stage are used, a heavier dose will be given in the induction stages and the control bracket reached earlier.

COMPLICATIONS OF ANTICOAGULANT THERAPY

Complications of anticoagulant therapy are almost exclusively those of hemorrhage. The kidneys and the lymphatic tissues are, for the most part, the most susceptible to changes and animals poisoned with prothrombin depressant drugs show hemorrhage first in the kidneys and lymphatic tissues. Bleeding from the bowel, nose, gums, and into the skin may also be seen in patients with severe depression of their prothrombin and allied factors. When the results of the original two-stage or TAME procedures are below 10 per cent, our laboratory routinely provides a one-stage determination. Experience has shown that a prothrombin below 10% as measured by the two-stage or TAME assay is not necessarily hazardous unless the result of the one-stage is also below 10%. If these levels are not far below the 10% lower limit of the therapeutic brackets, it is customary to withdraw the drug and wait until the prothrombin rises. If mild bleeding occurs, this same routine may be followed. If there is more than mild hemorrhage, it may be wise to give 10 to 15 mg. of Mephyton by mouth, and thus titrate the prothrombin back to the therapeutic bracket. It should be emphasized that thrombosis

may be considerably more important than mild hemorrhage and that large doses of Mephyton, rapidly to elevate a prothrombin that is not too far below 10%, may be more hazardous than mere withdrawal of the drug. Furthermore, large doses of Vitamin K often render the patient resistant to further anticoagulant therapy and, thus, the therapeutic program is further complicated.

LONG TERM ANTICOAGULANT THERAPY

The clinician is more and more frequently confronted with the decision as to whether or not to give a patient anticoagulants beyond his hospital stay. Indications for long-term therapy are quite definite in some conditions and less so in others. Since long-term therapy with heparin is for the most part impractical, it is the prothrombin depressants with which the clinician is chiefly concerned in prolonged anticoagulant administration.

At the present time it is generally accepted among authorities that, except for the possibility of hemorrhage, there are no deleterious effects of long-term anticoagulant therapy. Our own series of cases includes patients who have been on continuous therapy for more than sixteen years with no apparent ill effects. To be safe and effective, such therapy requires a thorough knowledge of the principles involved and complete cooperation between physician and patient.

FACTORS INFLUENCING UNIFORMITY OF CONTROL

In addition to the factors discussed earlier, under "Methods of Control", there are a number of others which may influence prothrombin levels. Among these are liver function, general nutritional state, diet, other drugs, alcohol, patient cooperation, individual tolerance, and, lastly, the experience of the clinician with the particular anticoagulant.

Poor liver function and/or nutritional deficiency usually render the patient more tolerant to the effects of a prothrombin depressant and less responsive to Vitamin K administration. A diet poor in proteins appears to be associated with frequent variation in prothrombin levels. On the other hand, a diet rich in leafy green vegetables, and particularly a supplemental vitamin capsule containing Vitamin K, will often increase the tolerance to an anticoagulant.

The methylxanthines, caffeine, and, more recently, some of the "tranquilizing drugs" have been found to increase the tolerance to prothrombin depressants. Contrariwise, the salicylates (which break down to coumarin-related substances) may precipitate a drop in prothrombin levels. Patients should be cautioned against the taking of more than 15 grains (0.97 gm.) of salicylate a day without first consulting their physician. Several of our patients have developed severe hypoprothrombinemia and hematuria as a result of excessive salicylate intake during upper respiratory infections. Alcohol elevates prothrombin levels, and patients who wish to use alcoholic beverages during a program of anticoagulant therapy must be advised to take the same amount daily in order that their anticoagulant intake may be adjusted to it.

It goes without saying that complete patient cooperation is essential to properly controlled anticoagulant therapy, and, the greater the experience of the clinician with the various drugs the more uniform will be the control. In general, the drug with which the clinician is most familiar is the one he should use.

Some patients appear to have idiosyncrasies to certain drugs, and when, despite a careful check of the above factors, a patient's prothrombin continues to be difficult to control, a shift to another anticoagulant will often remedy the difficulty.

COUNTERMEASURES

It may be necessary at times to neutralize rapidly the effects of anticoagulant

drugs. This may be as a result of hemorrhage, trauma, or required surgery.

If the hemorrhage is not severe, i.e., is limited to hematuria, gross or microscopic, and/or mild bruising, and if the prothrombin is above 5% by the one-stage method, withdrawal of the drug may be all that is necessary. This is particularly true if the drug is one characterized by a short recovery period. The patient, if not in the hospital, is instructed to collect single urine specimens and line them up along a window sill. Every 12 hours he reports their appearance, and, if consecutive specimens are deepening in color, another prothrombin determination is carried out. If this indicates a lower level than the previous one, a small amount (10 mg.) of Vitamin K may be given by mouth or vein and the urine observed during the subsequent 12 hours. This will usually prove effective, though of course more Vitamin K is indicated if it does not. As a rule, Vitamin K will not be necessary, and the urine will begin to show less blood shortly after the anticoagulant is withdrawn. This is particularly true if one of the drugs of Group 2 (those with short induction and short recovery periods) is being given. Therapy should be resumed cautiously when the urine is completely free of blood.

DECONTROL

The decontrol of anticoagulant therapy ordinarily presents no problem. Since thrombosis has occurred in some in-

stances when the drugs have been suddenly withdrawn, it is usually safer to discontinue them gradually. This provides for a slow return to normal prothrombin levels, and the latter may be checked at monthly intervals for a two-month period.

Of particular importance is the technique of decontrol of a patient with atrial fibrillation and a history of embolism. Such patients may require surgery, and, unless it is of an emergency nature, the decontrol should be gradual and completely covered by supplemental intravenous heparin given at four-hour intervals while the decontrol is in progress. Once daily the interval is extended to six hours and a prothrombin assay made at the end of that time. Surgery can usually be carried out when the prothrombin has reached 50% or more, but the heparin is continued until four hours prior to surgery. (In this connection the one-stage assay is a better indicator of possible bleeding than the two-stage methods since it leaves a greater number of factors, both clotting and anticlotting, uncontrolled). Heparin may usually be restarted during surgery in small amounts (30 mg. (3000 units) given intravenously over each six hour period) and, six hours following surgery, the regular therapeutic levels may be instituted. Prothrombin depressants should be restarted when the patient can take them by mouth. We have seen fatal emboli when such a routine has not been followed.



REFERENCES

1. Morawitz, P.: Die Chemie der Blutgerinnung. *Ergebn. Physiol.* 4: 307, 1905.
2. Lee, R. E., and P. D. White: A Clinical Study of the Coagulation Time of Blood. *Am. J. Med. Sci.* 145: 495, 1913.
3. Quick, A. M., Margaret Stanley-Brown, and F. W. Bancroft: A Study of the Coagulation Defect in Hemophilia and in Jaundice. *Am. J. Med. Sci.* 190: 501, 1935.
4. Koppel, J. L., P. M. Arscott, L. H. Friedman, and J. H. Olwin: Differences in Tissue Thromboplastins and their Influence on the Control of Anticoagulant Therapy. *Surgery* 43: 94, 1958.
5. Ware, A. G., and W. H. Seegers: Two-stage Procedure for the Quantitative Determination of Prothrombin Concentration. *Am. J. Clin. Path.* 19: 471, 1959.
6. Arscott, P. M., J. L. Koppel, and J. H. Olwin: Experience with the TAME Assay During Anticoagulant Therapy. *J. Lab. Clin. Med.* 51: 805, 1958.
7. Flynn, J. E., and E. T. Standley: On the Conversion of Prothrombin to Thrombin. *Trans. II. Conf. Blood Clotting a. all. Probl., Josiah Macy, Jr., Foundation.* 145, 1949.
8. Olwin, J. H.: Control of Dicumarol Therapy. *Am. J. M. Sc.* 217: 427, 1949.
9. Sise, H. S., S. M. Lavelle, D. Adamis, and R. Becker: Relation of Hemorrhage and Thrombosis to Prothrombin During Treatment with Coumarin-type Anticoagulants. *New Eng. J. Med.* 259: 266, 1958.
10. Hough, D. A., L. V. Lyons, J. L. Koppel, and J. H. Olwin: A Spectrophotometric Modification of the TAME Assay for "prothrombin": It's Use for the Control of Anticoagulant Therapy. *J. A. M. A.*, 188: 207, 1964.

ABSTRACTS AND REVIEWS

OF PUBLICATIONS BY THE STAFF

Treatment of Propoxyphene Poisoning. C. L. SWARTS, *Amer. J. Dis. Child*, 107:177-179, 1964.

Case report. A 5-year-old boy appeared ill when his mother woke from a nap. Five minutes later, the child went into a generalized convulsion. At examination in the home, he was deeply comatose and pale except for cyanosis of the nail beds and lips. Respiration was irregular, pulse rate high, and blood pressure low. Knee jerks were hyperactive, but all other reflexes were depressed. Pupils were fixed and reduced to pinpoint size.

Treatment. Apnea and deep cyanosis were reversed by intravenous or intracardiac epinephrine several times before the patient reached the hospital. The upper airway seemed to be obstructed, and both mouth-to-mouth breathing and intermittent positive-pressure resuscitation failed to improve his respiration. In the hospital, however, an orotracheal tube with an intermittent positive-pressure valve attached was passed, and cyanosis disappeared as adequate artificial respiration was achieved. When it was discovered that the boy had taken 9 capsules, 65 mg. each, of dextro propoxyphene hydrochloride (Darvon), nalorphine hydrochloride (Nalline) was given in a single intravenous dose of 2.5 mg. Within seconds the boy emerged momentarily from the coma and then lapsed again. The coma was lightened, however, and spontaneous breathing began. After gastric lavage, recovery was rapid.

Comment. The toxic depressing effects of propoxyphene have been reported before but are still little known. Both nalorphine and levallorphan tartrate (Lorfan) are rapid antagonists to respiratory depression caused by narcotics. They have potentially dangerous side effects, however, including the ability to cause respiratory depression themselves, and must be used cautiously. If this patient's ingestion of propoxyphene had been known earlier, a call to a poison control center would have eliminated guesswork and provided the correct treatment during the acute stage.

The Mallory-Weiss Syndrome. R. J. FREEARK, W. J. NORCROSS, R. J. BAKER, and E. L. STROHL: *Archives of Surgery*, Vol. 88, No. 5, pp. 882-887, May, 1964.

In 1929, Mallory and Weiss reported 15 patients with gastrointestinal bleeding following repeated emesis. During the past four years 13 patients have undergone surgical procedures for this syndrome.

The etiology as described by Mallory and Weiss suggests that the lacerating effect of the regurgitant jet of gastric contents against a closed cardia or esophagus persists as the most plausible explanation of the tears.

Although considerable controversy still exists as to the operative management of bleeding patients in whom the stomach and duodenum appear grossly normal, we have for some time advocated a long anterior gastrotomy before embarking upon empiric resections. A generous incision, 12 to 18 cm in length, from just above the pylorus to within 2 to 4 cm of the esophagus, is made midway between the greater and lesser curvatures. After the manual evacuation of clots and the introduction of angled retractors into the upper limits of the gastric incision, downward traction with the hand on the posterior wall of the stomach will usually permit visualization of the whitish mucous membrane which lines the esophagus. These maneuvers will usually visualize the Mallory-Weiss lesion when present and permit the suture repair needed for prompt hemostasis.

Our practice is to begin a catgut suture at the most distal extent of the tear and continue proximally a running locked stitch of generous depth until the edges of the defect are approximated.

We do not consider vagotomy necessary for the treatment of this syndrome. Insertion of a gastrostomy tube in eight patients provided postoperative decompression without the undesirable contact of a nasogastric tube along the site of operative repair.

Our single unfortunate experience with an empiric gastric resection which failed to control bleeding, and similar reports in the medical literature further emphasize the importance of searching for these lesions in all patients with unexplained gastrointestinal hemorrhage.

The prompt arrest of bleeding by direct suture repair through a generous anterior gastrotomy in 12 of 13 patients establishes the suture method as the treatment of choice for patients with continuing hemorrhage.

Periodic Evaluation of Outpatients. J. C. LASHOF and I. R. TURNER: *Archives of Environmental Health*, Vol. 8, April, 1964.

Two hundred patients past the age of 40 who had been attending the Health Center medical clinics for two years or longer had a repeat history and physical examination and the following tests—complete blood count, serologic test for syphilis, two-hour post prandial blood sugar, urinalysis, chest x-ray, electrocardiogram, proctoscopy, and Papanicolaou smear on female patients.

New diagnoses were made in 89 of the 200 patients studied. There were two types of disease diagnosed; asymptomatic, which could not have been diagnosed without laboratory studies, and, symptomatic, which was discovered by the more thorough history obtained during the re-evaluation.

The history, physical examination and two-hour post prandial blood sugar were the most productive procedures. It was not possible to assess fully the usefulness of some of the diagnostic procedures since not all abnormal findings were completely evaluated.

The study demonstrated the value of a periodic health examination. It also demonstrated the need for periodic re-examination of the outpatient clinic as well as of the out-patient. Since the beginning of the study, methods and systems of the clinics are under examination and new methods of quality control are being introduced to improve further the quality of medical care in the Health Center.

Mechanics of Breathing: Compliance and Resistance in the Normal Lung and Thorax. R. W. CARTON, and J. T. SHARP, In: *Advances in Cardiopulmonary Diseases*, Vol. II, Year Book Publishers, Chicago, 1964. p. 54.

This chapter reviews the subject of pulmonary mechanics. The mechanics of the lung and thorax are treated individually, and then combined, to give a total description of the mechanical properties of the pulmonary system. Methods by which the behavior of the lung can be carried back to the physical properties of its constituent tissues are described.

Estimation of Tissue Elasticity of the Lung. R. W. CARTON, J. W. CLARK, J. DAINAUSKAS, and A. BARRON, *J. Appl. Physiol.* 19:2: 236-242, 1964.

Theoretical work-volume curves for liquid-filled lungs were constructed based on an assumed geometric form and deformation of the terminal air spaces and on the characteristics of single fibers of elastic tissue. These curves were compared with those measured in rats and with one taken from the journal literature in the case of a human lung. The form of the measured curves fitted the theoretical curves. The assumptions necessary were considered in detail. In both species more elastic tissue has been found present in the lung than would be expected theoretically. The action of additional restraining elements was thought necessary at high volume.

Autoprothrombin C from a Commercial Thrombin Product. E. R. COLE, J. L. KOPPEL and J. H. OLWIN. *Nature* 202: 301, 1964.

Parke, Davis and Company Thrombin Topical of bovine origin was found to be a good source of autoprotehrombin C. Applying a procedure described earlier for the purification of autoprotehrombin C from activated purified prothrombin, autoprotehrombin C could be separated from commercial thrombin preparations. The product obtained was still

grossly impure although it was free of thrombin. Autoprothrombin C was capable of activating purified prothrombin only in the presence of calcium ions, phospholipids, and a source of factor V.

It is readily apparent that the use of commercial thrombin preparations in blood coagulation studies may result in misleading observations due to the inadvertent addition of the powerful procoagulant autoprothrombin C. The information presented in this report will make it possible to obtain both thrombin and autoprothrombin C concentrates which are, at least, free of each other.

Spectrophotometric Modification of the TAME Assay for "Prothrombin." Use for Control of Anticoagulant Therapy. D. A. HOUGH, L. V. LYONS, J. L. KOPPEL and J. H. OLWIN. *J. Am. Med. Assn.* 188:207, 1964.

This paper describes details of a new "prothrombin" assay and reports results with its use in the control of anticoagulant therapy in 1,414 patients. The assay is a spectrophotometric modification of the original TAME procedure and is based on the esterolytic action of thrombin on p-tosyl-l-arginine methyl ester (TAME). The assay was performed in parallel with the one-stage, two-stage, and Thrombotest assays in patients during anticoagulant therapy induction, maintenance (long and short-term), and recovery periods, the results closely paralleling those of the two-stage procedure. Because of (1) the uniform reproducibility of its results, (2) the simplicity with which it can be performed, and (3) its ready adaptability to routine clinical use, the authors have found it the most satisfactory "prothrombin" assay they have used.

Peripheral Arterial Disease. G. DE TAKATS, *Current Therapy* 1964, pp. 168-173. Published by W. B. Saunders Company, Philadelphia.

The treatment of peripheral arterial disease revolves around the recognition that arteries are contractile, that they carry liquid blood which may clot and that the arterial wall may be injured, inflamed or degenerate. In the latter case the vessel may become narrow or dilate, rupture or close. All these situations call for emergency or elective treatment.

Congenital vascular anomalies, arterial injuries, acute arterial occlusions consisting of embolism and acute thrombosis have been discussed. In addition, Raynaud's syndrome is described. The group of arterial inflammatory disorders, of which thromboangiitis obliterans is the most prominent example, are described. Arteriosclerosis, which is a general term, is discussed and the point is made that even though symptoms of arterial occlusive disease are limited to the extremities, one must take into account, that of a large number of patients presenting intermittent claudication, more than 50% died of myocardial infarction and very few of them, not more than 8%, come to amputation.

Various methods of surgical therapy are discussed in addition to vasodilators, anticoagulants, diet and abstinence from tobacco.

The Natural History of the Renal Manifestations of Systemic Lupus Erythematosus. V. E. POLLAK, C. L. PIRANI, and F. D. SCHWARTZ, *J. Lab. Clin. Med.* 63:537-550, 1964.

Serial clinical observations and histologic studies were made on the kidneys of 87 patients with systemic lupus erythematosus over periods ranging from 7 months to 8 years. A total of 176 renal biopsy and necropsy specimens were analyzed in detail by a semiquantitative method, and the histologic findings were classified in the following four groups: normal kidney, lupus glomerulitis, active lupus glomerulonephritis, and membranous lupus glomerulonephritis. Forty patients in whom the initial findings were normal kidneys, lupus glomerulitis and membranous lupus glomerulonephritis were followed for 160 patient-years. During this period only 2 developed mild lupus glomerulonephritis which responded to treatment, and 2 others developed severe disease and died in renal failure. Progression from the milder forms of renal involvement to severe active lupus glomerulonephritis was uncommon. Most patients with active lupus glomerulonephritis died in renal failure, but the clinical and histologic progression of the disease was slowed or halted in many by prolonged treatment with large doses of prednisone.

The Renal Glomerular Lesions of Pre-Eclampsia: Electronmicroscopic Studies. C. L. PIRANI, V. E. POLLAK, R. LANNIGAN, and G. FOLLI, *Am. J. Obstet. & Gynec.* 87:1047-1070, 1963.

Detailed electron microscopic studies were made of the glomerular lesions in renal biopsy specimens from 8 patients with pre-eclampsia. Serial studies were done on 3 patients.

Significant changes were observed in the endothelial, epithelial and axial cells of the glomerular capillaries. Much less pronounced changes were observed in the capillary basement membrane. Thus all elements of the glomerular tuft contributed in varying degrees to the abnormalities seen. No lesions were found in the peritubular capillaries. The complexity and variability of the pre-eclamptic glomerular lesions are emphasized, and some of the possible functional implications are discussed.

Bleeding Tendencies in the Surgical Patient: Their Detection and Correction. J. H. OLWIN and J. L. KOPPEL. *Surg. Clin. of N.A.* 44:185, 1964.

With the advance in knowledge concerning blood groupings and the widespread establishment of blood banks, the hazards to the surgical patient from severe blood loss as such have all but disappeared. The improvements in surgical techniques and the development of more radical procedures have themselves, however, necessitated the use of larger amounts of blood and this in itself has contributed to some of the bleeding problems confronting the surgeon.

In this paper the various types of bleeding problems which may confront the surgeon are outlined, methods for identifying hemostatic abnormalities prior to surgery are described, and procedures for the prophylactic and active treatment of hemorrhage in the surgical patient are suggested.

Although whole blood and plasma represent the main source of coagulation factors for the treatment of various hemorrhagic diseases, the indiscriminate use of blood or plasma transfusions in patients with bleeding tendencies is discouraged, particularly when it is intended to prevent rather than arrest hemorrhage. The potential danger of transmitting homologous serum hepatitis should always be considered, as should be the possibility of sensitization of the patient against the very coagulation factor he does not possess.

Phleboembolism in Orthopaedic Trauma. J. H. OLWIN and J. L. KOPPEL. *Clin. Orthopaedics* 28:89, 1963.

The problem of phleboembolism is as important to the orthopaedist as any complication with which he has to deal. Despite the trend toward early ambulation and the use of antibiotics, blood and anticoagulants, the incidence of this condition has not diminished materially.

In this paper an attempt has been made to present the principles and the practical considerations involved in the prophylactic and active treatment of phleboembolism in the orthopaedic patient. The discussion is centered primarily around the use of anticoagulant therapy and includes a brief explanation of the mechanism of blood coagulation as it is understood today, of various types of anticoagulants and their mode of action and of the factors to be considered in the choice of drugs. Methods for the administration and control of anticoagulants, for the routine of induction and maintenance of therapy and for counteracting the effects of anticoagulant drugs are also discussed. In addition, suggestions for treatment are outlined under conditions where anticoagulants may be contraindicated or ineffectual.

Mesenchymal Activation. G. M. HASS. In: *Evolution of the Atherosclerotic Plaque.* University of Chicago Press, Chicago and London, pp. 225-235, 1963.

Current experiments with combinations of these two forms of arterial disease are being prolonged beyond 1-2 years. It is too early to make anything more than a preliminary statement. It is clear that lamination of xanthomatous and a more normal fibrocellular

mesenchyme in atheromatous plaques occurs without any consistent change in experimental conditions. It seems, however, that, as the intimal plaques increase in age under the continuing stimulus of vitamin D, they acquire calcium in increasing amounts. There is evidence that this matrix mineralization in the intima stimulates the local penetration of vascularized medial granulation tissue into the intima. There is no evidence that "foam cell" deterioration and lipid accumulation alone will elicit this vascularization. As the intimal calcification and subsequent vascularization proceed, there is an increased incidence of mural fibrinous occlusive thrombosis, but the mechanisms involved are not yet clear. It may be that something more than merely lipids and calcium in a vascularizing degenerate intima provides the soil for thrombosis. The accumulating data, however, lead to the tentative conclusion that the process of aging, either of the atheroarteriosclerotic lesions or of the animal with the lesions, creates a situation that is increasingly favorable to the development of mural fibrinous thrombi on the lesions.

Modification of Homologous Tissue Reactions in Rabbits Pretreated with Donor's Cultured Fibroblasts. C. W. MONROE, R. H. ANDRESEN, and D. A. MADDEN, *Federated Procedures*, Vol. 23, No. 2, p. 452, 1964.

It was always possible to modify the classical tissue reaction to homologous grafts in rabbits by intraperitoneal injection into the host of donor blood leukocytes prior to grafting. The present experiments were designed to determine whether other cells possess conditioning capacity similar to that of leukocytes. Fibroblasts released from Ivalon sponges which had been implanted in male donors were grown in tissue culture to purify the cell strains. Eighty male host rabbits received twenty million or more cells from 18, 32 or 61 day cultures. Thereafter, each host received a musculofascial graft from the donor of the cells. Two weeks later, microscopic study revealed a modification of the host reaction to the homografts in 50% of all three groups. Thus, fibroblasts grown in tissue culture for as long as 61 days, in some instances, possess a conditioning capacity similar to blood leukocytes.

Transplantation of Embryonic Tissues to the Pregnant Rabbit Uterus. R. H. ANDRESEN, C. W. MONROE and D. A. MADDEN, *Federated Procedures*, Vol. 23, No. 2, p. 352, 1964.

Embryonic tissues (14-15 days of age) following intramuscular injection into the pregnant host always show prompt vascularization and rapid though limited rate of proliferation. The present experiments were designed to show whether or not embryonic tissues could elicit a vascular response when injected into the pregnant rabbit uterus. Eighteen pregnant females were subjected to laprotomy at midgestation. In each animal, six to eight embryos were removed from one uterine horn. The embryos were pooled, crushed with Potter's rod and injected into the lumen and wall of the pregnant uterus at areas between the gestation sites of the opposite uterine horn.

Two weeks later the maternal hosts were sacrificed and appropriate tissues were taken for microscopic study. They showed that embryonic tissues transplanted to the lumen of the pregnant uterus did not survive. Also there was no tendency for embryonic tissues to proliferate within the wall of the pregnant uterus. These studies indicate that the vascularizing stimulus ordinarily possessed by embryonic tissues following transplantation is not effective when embryonic tissues are transplanted to the pregnant rabbit uterus.

Post-Surgical Endoscopic Problems of Congenital Esophageal Atresia. P. H. HOLINGER, and K. C. JOHNSTON*, *Annals of Otology, Rhinology and Laryngology*, Vol. 72, No. 4, 1035-1050, December, 1963 (*deceased).

There are a number of recurring problems following surgery for congenital esophageal atresia and tracheoesophageal fistula that require endoscopic management. Some are strictly laryngeal, some tracheobronchial or esophageal, and others are combined broncho-esophageal problems associated with the anomaly or with the corrective surgical procedures. Analysis may aid in the prevention, diagnosis or treatment of some of the complications.

One hundred and twenty-nine infants with congenital esophageal atresia with or without fistula were observed on the endoscopic service of The Children's Memorial Hospital, in the

15 years between 1942 and 1962. One hundred and nine had an upper esophageal segment atresia and lower segment tracheoesophageal fistula; ten had an atresia of both segments; nine had a tracheoesophageal fistula without atresia; one infant had an esophageal atresia with a tracheoesophageal fistula between each segment.

Laryngeal problems were associated congenital anomalies, paralyses and post-operative edema. Eleven infants had laryngeal paralysis; six were bilateral, two right cord and three left cord paralysis, the latter post-surgical. Laryngeal paralysis invariably increases the difficulties of the post-operative period.

Tracheal problems occur at the site of the fistula leading to the lower esophageal segment. Obstruction due to inversion of the edges, granulomas and too tight a closure was present in 14 infants and was extremely serious. Diverticula consisting of a residual piece of the lower esophageal segment not completely removed were found in 7 infants. Bronchial secretions, chemical bronchitis, atelectasis and bronchiectasis constitute the bronchoscopic problems.

Esophageal strictures were present in 79 of the 129 infants, 69 at the direct end-to-end anastomosis site, 8 at the esophagogastric,—colic, or—jejunal site and 2 at the colo-gastric site. Early recognition and treatment of the narrowing lumen in the immediate post-operative period is stressed. Swallowing function is usually normal until semi-solids are added to the infant diet, at which time the anastomosis stricture is already firm and tough.

Foreign bodies were common endoscopic problems in infants or children who have had surgical reconstruction of the esophagus for congenital esophageal atresia.

Clinical and Laboratory Evaluation of Idoxuridine (IDU) Therapy in Herpes Simplex Keratitis. R. C. EY, W. F. HUGHES, A. W. HOLMES and F. DEINHARDT, *The Archives of Ophthalmology*, Vol. 71, pp. 325-331, March, 1964.

In 1960 Tamm reported that inhibitors of DNA synthesis could suppress the multiplication of herpes simplex virus in tissue culture. The treatment of herpes keratitis with idoxuridine (5-iodo-2-desoxyuridine, IDU) however, was first suggested by Kaufman. He and other authors have reported their generally good results in subsequent communications in experimentally induced disease in rabbits as well as in the naturally occurring infection in man. Attempts to repeat this work yielded somewhat less encouraging results in our hands and, therefore, a more extensive investigation was initiated. Our studies reported in this paper include: (1) the effect of idoxuridine on herpes keratitis in rabbits and patients, correlated with the presence or absence of demonstrable virus; (2) the influence of the immune status of rabbits on the clinical and virological course of the disease; and (3) the influence of idoxuridine on virus and host cells in a tissue culture system.

Our results indicate that idoxuridine (IDU, 5-iodo-2-desoxyuridine) (1) has no effect on herpes virus itself; (2) inhibits virus and cell multiplication to the same extent and, therefore, is not a specific antiviral agent; (3) has little beneficial effects in rabbits, whereas the immune status governs to a large extent the fate of the inoculated virus as well as the course of the disease; and (4) has little or no effect on established stromal disease in humans.

Although many dendritic ulcers eventually heal on idoxuridine therapy, complications are not infrequent and residual nebular opacities usually result. For a final evaluation of idoxuridine therapy, cases must be compared to a control series and to those with other methods of treatment.

Trans-Septal Catheterization of the Left Atrium: Report of an Unusual Complication. A. SUSMANO and R. A. CARLETON. *New England Jour. of Med.*, Vol. 270, No. 17, April, 1964.

The trans-septal needle in this procedure had previously been used approximately ten times without difficulty. The needles are sharpened approximately every two weeks and are carefully inspected at this time. The break in the needle tip occurred 1 mm. distal to the welded joint that is made between the 18-gauge tip. It is assumed that this break represented the result of repetitive flexing, with eventual metal fatigue.

The orientation of the fragment in the liver suggests the possibility that it was carried to the liver via the hepatic artery. In this event, one would postulate that the tip of the

needle had entered the left atrium and had been sheared at the atrial septum. Alternatively, the needle may have broken in the right atrium and made its way, even in a supine patient, into the hepatic venous system.

This and other cases demonstrate that trans-septal catheterization of the left atrium is not without hazards. Hemopericardium of varying severity due to perforation of the free atrial wall has been reported, as has penetration of the ascending aorta.

Forty-nine trans-septal catheterizations performed in this laboratory have produced 3 episodes of pericardial tamponade, 1 of which required pericardiocentesis during the catheterization. In all other regards the trans-septal catheterization technic has proved satisfactory in our hands.

The durability of the trans-septal needles is not known. Certainly, the experience reported here suggests that extreme caution must be taken in the use of these needles and in their inspection before insertion.

Comparative Study of Patients with Pneumonia, Atelectasis, and Lung Infarction Following Inhalation of Various Gas Mixtures. M. L. BOGDONOFF, *Radiology*, Vol. 82, No. 4, 679-685, April, 1964.

Seventy selected patients in whom diagnosis of pneumonitis, atelectasis, or lung infarction was reasonably proved were examined by means of three serial roentgenographic studies:

- a. a preliminary film;
- b. an examination made after ten to fifteen minutes of inhalation of pure oxygen, 70 per cent helium-30 per cent oxygen, or 80 per cent helium-20 per cent oxygen, and
- c. radiographs obtained fifteen minutes after the second study, with the patient breathing room air.

No roentgenographic differences could be demonstrated in comparing films made after the inhalation of pure oxygen and those obtained after the breathing of room air.

When one of the helium and oxygen mixtures was employed, no serial radiologic changes were discernible in the three sets of radiographs of the 56 patients with pneumonitis or of the 6 with atelectasis.

Radiographs obtained following the inhalation of an 80 per cent helium-20 per cent oxygen mixture demonstrated relatively increased radiolucency in certain of the 8 cases of lung infarction during the first seventy-two hours of their evolution. These observations raise but do not establish the possibility that this approach may yield findings helpful in making an early diagnosis.

Biliary Tract Surgery in the Aged Patient. E. L. STROHL, W. G. DIFFENBAUGH, and R. E. ANDERSON, *Geriatrics*, Vol. 19, pp. 275-279, April, 1964.

Biliary tract disease is the most common pathological process requiring abdominal surgery in the patient over 70 years of age. We found that 1 patient in each 7 having biliary tract surgery was over 70 years of age.

Carcinoma of the gallbladder and bile ducts was present in 8.2 per cent of our patients over 70 years of age, an incidence three times as great as in those patients under 70 years of age.

The mortality rate in elective surgery was 6.1 per cent.

Nine of the 14 deaths in patients over 70 years of age were due to carcinoma, common bile duct complications, or acute calculous cholecystitis with gangrene and perforation.

Advanced age is not necessarily a deterrent to biliary tract surgery. Surgery, however, should be carried out before serious complications of biliary tract disease develop. The tragedy of biliary tract surgery is related to neglect in most instances.